OPTIMISATION OF ULTRAFILTRATION FOR HUMAN SERUM ALBUMIN AT NBI

by

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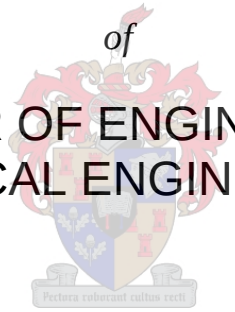

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ABSTRACT

National Bioproducts Institute (NBI) is a key plasma fractionator within the Southern African countries. Human serum albumin (HSA), is the most abundant plasma protein in human blood and has significant physiological and therapeutic benefits. The global demand for HSA is steadily increasing and this has compelled plasma fractionators to optimize the key processes that are used to manufacture this plasma derived medicinal product (PDMP). A key process in the manufacture of HSA is the use of ultrafiltration (UF), a key membrane separation technology, to remove unwanted salts and solvents from the dissolved active pharmaceutical ingredient (API). UF of protein solutions consists of several key steps including first concentration step, diafiltration steps one to five and second concentration step. Ultrafiltration performance is limited by inherent process characteristics such as concentration polarization (CP) and membrane fouling (MF). During this study, the key factors that influence the UF of HSA namely protein concentration, ethanol concentration, temperature and ionic strength, were optimized within pre-defined ranges to evaluate their impact on permeate flux and membrane performance. Permeate flux is a key determinant of process time, which directly impacts annual production capacity with a particular UF unit (C200 UF rig). A key objective of this study was to maximize production capacity through optimization of the key factors above, using the C200 UF rig. The results of this study show that protein concentration is the key factor that influenced the various steps of UF of HSA and overall production capacity. The optimum protein concentration range for maximum productivity was 60.00g/L to 100.00g/L. The optimum protein concentration for UF of HSA was approximately 100.00g/L, determined using the gel concentration model. Ionic strength diafiltration diluent (1M – 3M) reduced permeate flux during UF of HSA. Ethanol (<10% v/v) also had a reducing effect on, permeate flux during UF of HSA, with the optimum range specified between 0% (v/v) to 7.75% (v/v). Optimum permeate flux is achieved at a temperature of 25°C for UF of HSA. The optimum protein concentration for UF is within the protein concentration range for optimum productivity. Further, the proposed increase in protein concentration during UF results in a greater than 30% increase when compared to the current batch size.

ABSTRAKTE

Die National Bioproducts Institute (NBI) is 'n belangrike bloed-plasma fraksioneerder in Suider-Afrika. Menslike serum albumien (MSA), is die plasma-proteïen in die grootste konsentrasie in menslike bloed, met beduidende fisiologiese en terapeutiese voordele. Die toenemende wêreldwye vraag na MSA het plasma fraksioneerders verplig om sleutel prosesse wat gebruik word om hierdie plasma-afgelei geneesmiddel te vervaardig, te verbeter. 'n Belangrike proses-stap in die vervaardiging van MSA is ultrafiltrasie (UF), 'n membraan-skeidingstegnologie wat gebruik word om ongewenste soute en oplosmiddels van die aktiewe farmaseutiese bestanddeel te verwyder. UF van proteïenoplossings bestaan uit verskeie opeenvolgende stappe, naamlik die eerste konsentrasie stap, diafiltrasie stappe 1 tot 5, en die tweede konsentrasie stap. Die werkverrigting van UF word tipies beperk deur meganismes soos konsentrasie polarisasie (KP) en membraan aankorsing (MA), inherent aan die membraan-filtrasie proses. In hierdie studie word die impak van die belangrikste proses-parameters op die UF van MSA proses ondersoek, naamlik proteïen-konsentrasie, etanol-konsentrasie, temperatuur en ioniese sterkte, binne ‘n vooraf-gedefinieerde operasionele gebied. Die impak hiervan op permeaat-deurvloei produksie-tempo en membraan prestasie is ëvalueer, met in agneming van KP en MA meganismes. Permeaat-deurvloei-tempo het ‘n kritiese invloed op die totale prosesseringstyd, wat 'n direkte impak jaarlikse produksie kapasiteit met 'n bepaalde UF eenheid het, in hierdie geval die C200 UF opstelling. 'n Belangrike doelwit van die huidige studie was om jaarlikse produksie kapasiteit te maksimeer, deur optimering van proseskondisies vir hierdie opstelling. Die eksperimentele resultate het getoon dat proteïenkonsentrasie is grootste invloed op die verskillende stappe in die UF van MSA, sowel as die jaarlikse produksie kapasiteit gehad het. Die voorkeur proteïen-konsentrasie vir 'n maksimum jaarlikse produksie kapasiteit was tussen 60 g/L en 100 g/L. ‘n Optimum proteïen-konsentrasie in hierdie gebied van 90 g/L tot 100 g/L, is bepaal deur toepassing van die konsentrasie polarisasie (gel konsentrasie) model. Aanpassing van die ioniese sterkte van die diafiltrasie verdunningsmiddel tot 1M-3M hetdie permeaat-deurvloei-temp verminder. Op soortgelyke wyse het toenames in die etanol tot by 10% (v/v) ook die deurvloei-tempo verminder, met ‘n verminderde effek in die voorkeur gebied van etanol konsentrasies tussen 0% (v/v) en 7.75% (v/v). Die voorkeur temperatuur vir maksimering van die permeaat-deurvloei-tempo was 25 °C, terwyl verdere toenames in temperatuur MSA-proteïen-onstabiliteit sal veroorsaak. Implementering van die voorgestelde verhoging in proteïen-konsentrasie tydens UF van MSA sal ‘n toename van meer as 30% in die grootte van ‘n

enkellading deur die proses bewerkstellig, met dienooreenkomstige toenames in die jaarlikse produksie-kapasiteit.

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LIST OF EQUATIONS

LIST OF SYMBOLS

α	Alpha
β	Beta
A	Area (m^2)
A_{280nm}	Ultraviolet Absorbance at wavelength of 280nm
Ɛ280nm	Sedimentation coefficient of HSA (5.8g/L)
J	Permeate flux (LMH)
J_w	Clean water permeate flux (LMH)
K	Mass transfer coefficient
L	Length (cm)
C_f	Concentration of solute in feed (g/L)
C_g	Concentration of solute in gel boundary layer (g/L)
C_b	Concentration of the solute in the bulk solution (g/L)
C_p	Concentration of solute in permeate (g/L)
ΔP	Transmembrane pressure (Bar)
$\Delta\pi$	Osmotic pressure difference
μ	Solution viscosity (cPa)
Q	Flow rate (L/h)
R_T	Fouling resistance
R_m	New membrane resistance
R_a	Concentration polarization resistance
R_g	Cake layer resistance
b	Fouling rate
L_p	NWP after cleaning
$L_{p,0}$	Initial NWP

ABBREVIATIONS

ANOVA	Analysis of Variance
API	Active Pharmaceutical Ingredient
BSA	Bovine Serum Albumin
CCD	Central Composite Design
CPP	Critical Process Parameters
CQP	Critical Quality Attributes
CVD	Constant Volume Diafiltration
CP	Concentration Polarization
DOE	Design of Experiments
ETOH	Ethanol
EDTA	Ethylenediaminetetracetic
ELISA	Enzyme Linked Immunosorbent Assay
GEP	Good Engineering Practise
GMP	Good Manufacturing Practice
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HPTFF	High Performance Tangential Flow Filtration
HSA	Human Serum Albumin
IEP	Isoelectric Point
MF	Membrane Fouling
MWCO	Molecular Weight Cut Off
NaCl	Sodium Chloride
NaOCl	Sodium Hypochlorite
NaOH	Sodium Hydroxide
NBI	National Bioproducts Institute
NF	Nanofiltration
NFF	Normal Flow Filtration
NWP	Normalized Water Permeability

OFAT	One-Factor-At-A-Time
O/N	Overnight
PDMP	Plasma Derived Medicinal Product
PES	Polyethersulfone
PIR	Pressure gauge
ppm	Parts per million
PPT	Precipitate
RO	Reverse Osmosis
SADC	Southern African Development Community
SANBS	South African National Blood Transfusion Service
TCF	Temperature correction factor
TFF	Tangential Flow Filtration
TMP	Transmembrane Pressure
UF	Ultrafiltration
V	Tube clamp or valve
WBTS	Western Province Blood Transfusion Service
WHO	World Health Organization
WFI	Water for Injection

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1 INTRODUCTION

Human serum albumin (HSA) is the most abundant plasma protein in human blood and functions as a carrier of steroids, fatty acids and some hormones. It is a lifesaving plasma protein used in the treatment of shock, thermal injuries (e.g. burns), therapeutic plasmapheresis and restoration/maintenance of circulating blood volume (Hastings and Wolf, 1992; Liumbruno *et al.*, 2009). The ability of HSA to act as a drug transfer molecule due to its high ligand binding affinity and especially high plasma concentration (5g/100mL) has long been established through various studies (Bosse *et al.*, 2005; Sugio *et al.*, 1999). Most recently, several investigations have shown the potential of this plasma protein to bind and transfer other beneficial drugs including the hepatitis B drug, adefovir dipivoxil (Shahabadi *et al.*, 2015); stilebene compounds (Nair, 2015) and Z-ligustilide (Chen *et al.*, 2015). During the period from 1950 to 1960 HSA was the key market driver for the plasma derived medicinal products (PDMP) industry; in the subsequent years HSA was superseded by polyvalent immunoglobulin as the key PDMP (Robert, 2009). As a renowned "transport plasma protein", HSA is fast re-establishing itself as the key market driver in the plasma fractionation industry.

The global demand for PDMP's has shown a consistent growth rate of approximately 13% per annum, for the period 2002 to 2009 (Robert, 2011) and this trend has been maintained through to 2015. Further, the marketing research bureau has estimated that the worldwide plasma derived products industry is worth in excess of $11.5 billion, with albumin sales staking a claim of 10% of the total market (Robert, 2011). The African and Asian markets show the most potential for the sale of PDMP's. The National Bioproducts Institute (NBI), a key fractionator within the Southern African countries, is ideally positioned within the African market to satisfy the demand for PDMP's within this region.

The market demand for HSA, driven by the therapeutic benefits of this drug, including its use as a novel drug delivery system, will ensure that this plasma derived product is a key contributor to the global plasma market. It is therefore essential that organizations that produce HSA continuously improve their production processes, in an effort to satisfy the ever increasing demand for these lifesaving drugs. NBI manufactures HSA according to the methods of plasma fractionation defined by Kistler and Nitschmann (1962). These plasma fractionation methods will be discussed in detail in chapter two. Briefly, this

manufacturing process can be classified into two phases. In the first phase (Figure 2:3), Fraction V paste containing HSA is separated from a starting pool of human blood plasma by exploiting temperature, pH, ethanol (ETOH) concentration and protein concentration. This plasma fractionation process also separates various other fractions that contain plasma proteins e.g. Fraction II paste which contains immunoglobulin. Various solid-liquids separation techniques such as centrifugation and filtration are used to separate the protein fractions. The second phase (Figure 2:4) in the production of HSA involves the dissolution of a fixed mass of Fraction V paste in a water for injection-ethanol (WFI-ETOH) admixture. HSA in the dissolved Fraction V paste is purified through the processes of depth filtration and ultrafiltration (UF), formulated (addition of stabilizers) and dispensed into final product containers. Although, the first stage in the manufacture of HSA is well documented and optimized according to protein yields and purity, the second stage in this process (purification, which includes dissolution of Fraction V paste containing HSA and subsequent refining of HSA) can be optimized further by evaluating the effects on process performance of key process parameters.

Ultrafiltration, a novel membrane separation technology, is a key unit operation in the purification of HSA, where UF epitomizes the need for removal of unwanted impurities, solvents and other molecules. UF as a unit operation generates an active pharmaceutical ingredient (API), which is HSA, at specified protein concentration, ready for final filling and parenteral use. Following a careful analysis of all the unit operations involved in the purification of HSA, UF bears the greatest expense due to the high membrane costs, investment of capital for equipment and extended man hours. These costs can be reduced by optimization of the UF process. The optimization of the UF of HSA will ultimately lead to decrease in costs and increase in productivity. A key process improvement point will be to maximize the mass of Fraction V paste dissolved during the purification of HSA (Figure 2:4), i.e. maximize the protein concentration at which UF of HSA is completed. One method of increasing process capacity is to increase the size of peripheral equipment, however, this may not be a feasible process improvement strategy since larger equipment generally requires increased capital expenditure. A more productive approach is to determine the range of protein concentration that favours the purification of HSA at optimum permeate flux. The impact of ionic solutions on permeate flux during UF of HSA has been investigated by several authors (Swaminathan *et al.*, 1981; Fane *et al.*, 1983; Lim and Mohammad, 2010). A key objective of this study is to determine the effect of

ionic solutions on permeate flux during UF of HSA, determine the nature of the effect and if permeate flux can be maximized by using ionic salt solution of certain concentration. NBI completes the UF of dissolved Fraction V paste containing HSA using WFI as a diafiltration diluent to maintain constant volume during diafiltration. A further optimization initiative will be to determine the optimum temperature at which to complete the UF of HSA since the impact of temperature on proteins and membranes is well documented and discussed further in chapter two.

This study will be dedicated towards the optimization of the UF of HSA by evaluation of key process parameters that govern this process, and thereby provide guidelines for process optimization and control. The guidelines for process design and control will be derived from process engineering principles as well as evaluating protein biochemistry at various steps in the process. The current UF process, as practiced by NBI, has the potential to increase productivity (kg HSA protein refined per year) by evaluating key factors that influence this process. Therefore, strategic optimization of the productivity of HSA will also be considered during this work.

1.1 MEMBRANE BASED SEPARATION TECHNOLOGY: ULTRAFILTRATION

Membrane based separation technologies have risen to prominence in the last 60 years and has become a suitable alternative to the traditional separation techniques such as distillation, precipitation and crystallization, in many industrial processes (Muralidhara, 2010). Membrane technologies are typically described as energy efficient with low environmental impact, high selectivity attributes, with the ability to produce increased yields of desired products at high throughput rates (Strathmann, 2005). However, the most appealing benefit of membrane based technologies to the engineering arena is the achievable separation efficiencies, when compared to conventional techniques, and the ease with which they can be integrated into various manufacturing processes (Saxena *et al.*, 2009).

Due to these attributes, membrane based technologies such as microfiltration and UF are used extensively in protein purification and separation processes. These pressure driven processes with membranes of specified molecular weight cut off (MWCO) points, are well suited to the high retention of the protein of interest whilst removing unwanted

macromolecules (Saxena *et al.*, 2009). Ultrafiltration is used in the processing of HSA, following the dissolution of Fraction V paste (containing HSA), to remove unwanted micromolecules (e.g. citrate and aluminium) and solvents (such as ETOH) to produce a PDMP's suitable for parenteral use. The key disadvantages of UF includes decrease in membrane performance due to membrane fouling (MF) and concentration polarization (CP), high capital costs for membranes, and the requirement for regular cleaning of membranes. It is therefore imperative to optimize the process parameters that govern MF and CP in an effort to decrease the overall processing costs for the UF of proteins, whilst maintaining the yield, safety, quality and efficacy of the HSA produced.

1.2 PROJECT AIMS AND OBJECTIVES

The project aim is to evaluate the factors that influence the permeate flux during the UF of HSA, and to provide guidelines for process design and control of this key unit operation. The key objectives in achieving the stated aim include:

- Determine the impact of protein concentration on the UF of HSA. This study will aim to determine the maximum protein concentration at which to complete UF of HSA. Further, this study will evaluate the effects on permeate flux with change in protein concentration. Establishing this optimum protein concentration has significant impact on the quantity of Fraction V paste that can be used per batch, which is key to increasing production capacity. This has significant impact on process improvement strategies and process control with respect to productivity i.e. kilograms of Fraction V paste processed per year.
- Determine the impact of an ionic solution (of a particular concentration), e.g. sodium chloride (NaCl) when used as a diafiltration diluent, on permeate flux during the UF of HSA. Currently, WFI is used as the diafiltration diluent during UF. Screening experiments and literature indicate that ionic solutions have an impact on permeate flux during UF. This study will attempt to evaluate the impact of ionic solutions of varying concentration, on permeate flux during UF of HSA. The ionic solution used in this study is NaCl.
- Determine the optimal processing temperature at which the UF of HSA can occur. Numerous authors have noted the impact of temperature in the UF of proteins and this study will aim to establish the optimum temperature for UF of HSA.
- During the refining of HSA, the Fraction V paste is dissolved in an admixture of WFI and ETOH, with ETOH having a final concentration of 10% (v/v) in the

dissolved Fraction V paste. This present study will determine the impact of ETOH concentration on permeate flux during UF of HSA.

1.3 DESIGN OF EXPERIMENTS

Design of experiments (DOE) utilizes statistical methods such as central composite design (CCD) to analyse and interpret key factors that influence product processes or product quality by conducting controlled, scientific based tests on the experimental unit (Telford, 2007). The statistical application of DOE in the pharmaceutical environment results in the scientific understanding of key factors that affect critical process parameters (CPP) and critical quality attributes (CQP) of products (Shivhare and McCreath, 2010). Thus the effect of CPP's and related impact on CQP allows the optimization of production processes leading to improvement in quality of products with a subsequent positive impact on market share, decreased costs and improved profits (Anderson and Kraber, 1999). The principles of DOE is utilized in a wide range of applications including optimal use of industrial equipment e.g. stationary hook hoppers (Kukreja *et al.*, 2011), improving tablet coating quality and performance applications (Porter *et al.*, 1997), and increasing the yields of pharmaceutical intermediate products (Wilson, 2012).

The methodology of DOE, which includes statistical techniques such as central composite design (CCD) encompasses a multi-factor approach to determine the effects on the experimental unit, in a limited number of runs through use of scientific and statistical techniques. Therefore, a key benefit of DOE is to determine critical factors that have significant impact on CQP or output variables in the shortest time, when compared to other experimental strategies such as one-factor-at-a-time (OFAT) experiments (Kukreja *et al.*, 2011). Another key advantage of DOE is that it allows the evaluation of interactive effects between factors that may not be evident in OFAT experimental methodologies (Anderson and Kraber, 1999). Further benefits of DOE include:

- Improve product design (Telford, 2007)
- Focal point for quality by design initiatives in pharmaceutical manufacturing by determining CPP (Shivhare and McCreath, 2010)
- Analysis of variability with respect to raw materials, operators and process variability (Shivhare and McCreath, 2010).

In this study, DOE was used to evaluate the active factors (protein concentration, temperature, ETOH concentration and ionic strength) at various levels, using the CCD statistical technique. Experimental runs were completed at the minimum and maximum start points as well as the centre points, for each factor. The output variable in this study was membrane performance which was defined by permeate flux during UF of HSA, membrane flux recovery and protein retention. Further, UF of HSA productivity was evaluated as a function of process time and other manufacturing constraints. The permeate flux data from all experimental runs was statistically analysed using the analysis of variance (ANOVA) technique, in particular the Fischer's Test. The relationship between predicted responses for each factor, across the selected level and the desired response (maximizing permeate flux during UF of HSA) is defined as the desirability function. Desirability is measured on a scale from 0 to 1 were 0 is an undesirable response and 1 is a desirable response (Asfaram *et al.*, 2015).

2 PLASMA FRACTIONATION AND PLASMA PROTEINS

2.1 INTRODUCTION

Human PDMP's such as HSA, Factor VIII, Factor IX and immunoglobulin are lifesaving, therapeutic drugs that are renowned for their treatment of chronic diseases and disorders, including haemophilia and Kawasaki disease (Hastings and Wolf, 1992; Berger, 2002). The Dublin Consensus Statement asserted that the plasma fractionation industry (including other sectors of the blood industry such as blood collection agencies) has a critical responsibility to provide a safe, sufficient and sustainable supply of PDMP's to the patients who depend on these lifesaving therapies (Mahony and Turner, 2010). The growing global requirements for safe (free of viruses and bacteria), therapeutically effective human PDMP's and the growing number of patients that require these therapeutic products has driven plasma fractionation organizations to continuously evaluate and improve related production processes.

The increasing demand for PDMP's coupled with a shortage of source raw material (i.e. human plasma) has compelled fractionation organizations to optimize manufacturing processes, to increase process capacity (i.e. productivity), product yields and product purity (Roberts, 2011). Process optimization is a value adding initiative, since it increases yield and process efficiency, whilst reducing process time and associated costs. The integration of new technology and improvement of current techniques is critical to the process optimization strategy. The increased demand for high quality, viral safe products and the increasing regulatory requirements towards sustainable good manufacturing practice (GMP) has ensured that the plasma fractionation industry continuously focuses on process improvement strategies.

NBI is one of South Africa's largest biopharmaceutical institutes specializing in the production of PDMP's. The key products manufactured by NBI include albumin, immunoglobulin and clotting factors which are all prepared as sterile, injectable solutions (parenteral solutions). NBI is committed to producing high quality blood plasma products by following the principles of GMP as well as Good Engineering Practise (GEP). In the South African market, the demand for HSA and immunoglobulin shows a similar trend to that represented in the global market – a steady year on year increase is projected until 2015 and beyond (Roberts, 2011; Roberts, 2009). Further, there is an

increasing need for plasma derived products to be supplied to the developing countries that form the Southern African Development Community (SADC) region. International competitors are evaluating business opportunities within the untapped, African plasma market and some organizations have already registered certain products for sale in South Africa. It is therefore imperative that the NBI optimize processes related to the manufacture of plasma derived products to ensure that supply and demand is satisfied and that NBI remains a market leader in Africa with respect to the production of plasma derived products.

A careful analysis (conducted by NBI) of the product profile, market requirements and process strategy for production of human PDMP's has resulted in the selection of two critical products (and related processes), i.e. HSA and immunoglobulin, as the focal point for capacity expansion and process optimization. The current process optimization study will focus specifically on the UF of HSA, since the upstream process of separating proteins into fractions of paste from plasma pools, using cold ETOH fractionation techniques, was optimized as part of an earlier initiative. Further, the UF of HSA was selected for optimization since currently accepted process improvement strategies can be used to improve product yield, process productivity (process time and capacity) and product purity. The inherent flux decline during UF of HSA, due to CP and MF, coupled to the denaturation of proteins observed during present industrial operations, have provided enough scope to warrant a full process evaluation and optimization study.

The critical goals in optimization of membrane process such as UF of proteins is to achieve a maximum permeate flux with maximum solute rejection and minimal operational and consumable costs with no negative impact on product yields or purity (Sablani *et al.*, 2001). The key process objectives of this study will be to increase productivity whilst maintaining or improving the current process yields and product purity. This study will also investigate the synergy between process parameters in achieving the stated objective and suggest an optimal process design that will also consider a possible trade-off between the objectives.

2.2 PLASMA FRACTIONATION AND THE GLOBAL DEMAND FOR PLASMA FRACTIONATED PRODUCTS

2.2.1 PLASMA AND PLASMA PROTEINS

Human plasma is a unique biological material and is described by the world health organization as the "liquid portion remaining after separation of the cellular components from blood" (Burnouf, 2012). The modernization of common protein identification techniques such as 2D-electrophoresis, mass spectrophotometry and antibody arrays has resulted in identification of approximately 300 proteins in human plasma – some of which have significant clinical value, e.g. HSA, immunoglobulin, protease inhibitors, Factor VIII and Factor IX (Burnouf, 2012; Anderson and Anderson, 2002; Putnam, 1975).

In a study conducted by Laub *et al.*, (2010) plasma recovered from remunerated and non-remunerated donors from several European countries, including Germany, France, Belgium and the United States of America, were analysed for total protein and key plasma protein markers such as albumin and immunoglobulin. This study confirmed that human plasma has a high total protein concentration of approximately 60g/L, with more than 90% (approximately 55g) of these proteins having recognized clinical and therapeutic significance (Burnouf, 2006; Laub *et al.*, 2010). This study also revealed two major plasma protein groups across all donor groups namely albumin (30-40g/L) and immunoglobulin (7-10g/L). Transferrin and fibrinogen have similar concentrations (2-3g/L) in human plasma and constitutes the third major group of plasma proteins (Burnouf, 2006; Laub *et al.*, 2010). Plasma proteins that have the lowest concentration in plasma include interleukin proteins (e.g. interleukin 6 concentration is 0-5 x 10^{-12} g/L) and tissue factors (Anderson and Anderson, 2002). Figure 2:1, derived from Putnam (1975), shows the maximum concentration (g/L) of key plasma proteins in 1L of human plasma. The concentrations of these plasma proteins listed herein are in close agreement with those listed by Burnouf (2006) and Laub *et al.*, (2010)

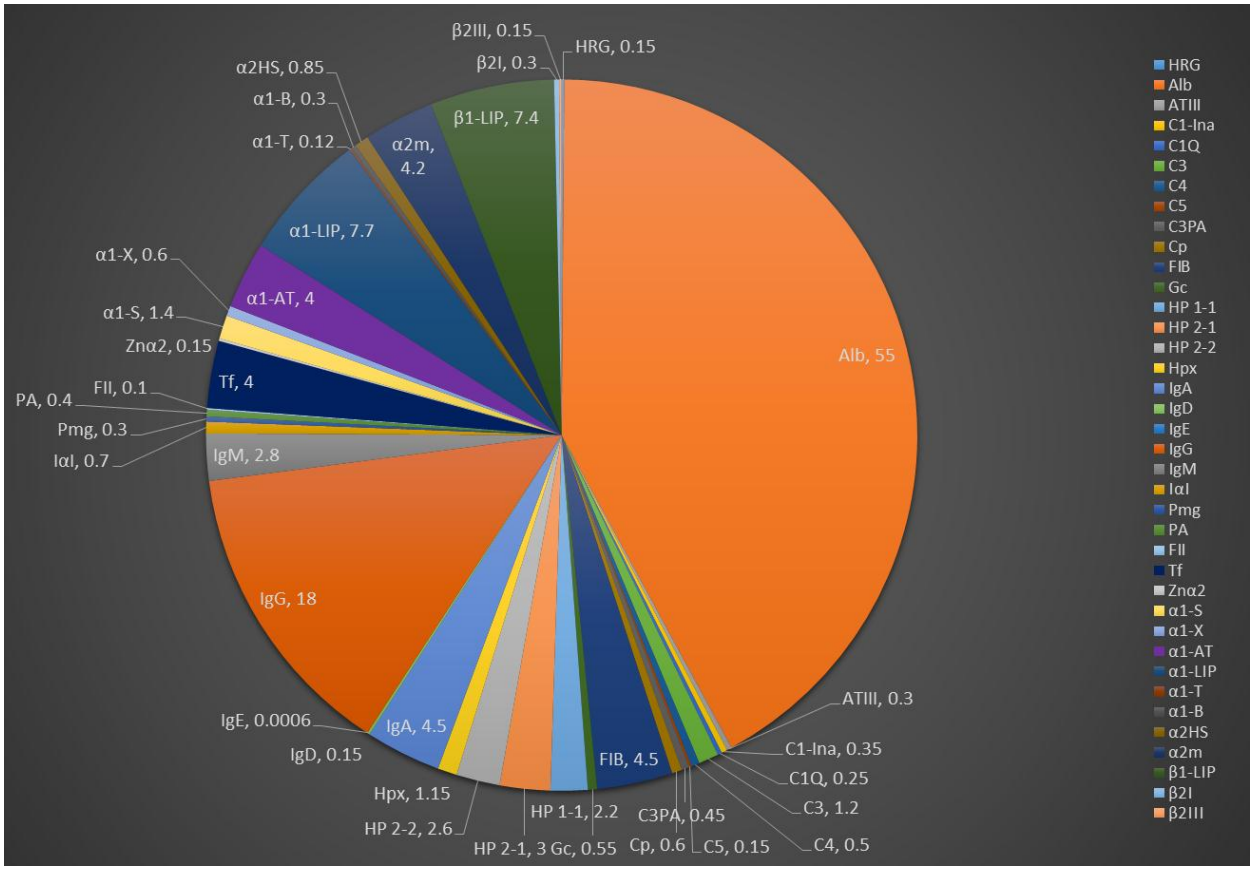


Figure 2:1: *Pie chart indicating the maximum concentration (g/L) of key plasma proteins found in human serum (Putnam, 1975). Albumin and immunoglobulin are the most abundant plasma proteins, whilst transferrin, glycoproteins and fibrinogen make up the third major class of plasma proteins.*

Human plasma is the unique, primary raw material used to manufacture essential protein based medicines (e.g. albumin, immunoglobulin), through a process called fractionation, which are used to treat various diseases and disorders including haemophilia A/B, primary immunodeficiency, renal failure and liver failure. In South Africa, the main source of plasma for fractionation is derived from non-remunerated, tested donors, who either donate whole blood (blood components, e.g. red blood cells, plasma are separated after collection of blood) or donate the plasma component of blood through a process known as apheresis (El-Ekiaby *et al.*, 2009). Blood and related components are collected by two stringently controlled and regulated national blood collection agencies namely the South African National Blood Service (SANBS) and the Western Province Blood Transfusion Service (WBTS). The viral safety of human plasma is critical to the use of this raw material to manufacture PDMP's. The identification and transmission of key pathogenic viruses such as human immunodeficiency virus (HIV), Hepatitis B virus (HBV) and Hepatitis C virus (HCV)

has forced the plasma fractionation industry to implement viral testing and removal strategies to ensure the safety of human plasma used to produce medicinal products (Burnouf, 2006; Burnouf, 2012). These viral safety strategies include:

- Comprehensive screening and testing of blood donors, which is normally conducted by the national blood collection agencies
- Viral testing of source plasma before use in fractionation processes using specialized diagnostic method such as the enzyme linked immunosorbent assays (ELISA).
- Viral reduction methods implemented in the production of plasma derived medicines, e.g. solvent detergent methods, pasteurization and nanofiltration methods (Burnouf, 2012).

Human plasma contains more than 300 different plasma proteins, some with important clinical significance. HSA is a key plasma protein derived from human plasma. The collection of plasma is stringently controlled due to the high risk of infection by pathogenic viruses such as HIV. Patient safety is a key component of the manufacture of PDMP's and the safety of the manufactured parenteral products are enhanced by ensuring that products such as HSA are manufactured from safe, raw materials (human plasma).

2.2.2 GLOBAL DEMAND FOR PLASMA DERIVED PRODUCTS

The global demand for plasma derived products has shown a consistent growth rate of approximately 13% per annum for the period 2002 to 2009, this trend is expected to be maintained through to 2015. The marketing research bureau has estimated that the worldwide plasma derived products industry is worth in excess of $11.5 billion, with intravenous immunoglobulin sales holding up to 46% of the market and HSA sales making up 10% of the total market (Robert, 2011). During the period 1950 to 1960, albumin was the key market driver for the PDMP's industry. During the late 1960's, the therapeutic potential of factor VIII and factor IX was discovered and these products superseded albumin as the key market driver for plasma derived products. By the mid 1990's polyvalent immunoglobulin superseded factor VIII and factor IX as the market driver for plasma derived products (Robert, 2009). The latest market research indicates

that HSA is not a key market driver in the plasma fractionation industry. However, the therapeutic value of this plasma protein coupled to its novel use in gene delivery systems ensures that albumin is a highly valued PDMP. This is further highlighted by the increasing worldwide demand for albumin, as depicted in Figure 2:2. Current figures compiled by the marketing research bureau showed a compounded annual growth rate for the demand of albumin of +4.8% since the year 2000. Further, in a presentation delivered by Patrick Robert (2014), at a global symposium on the future of blood and plasma donations, it was proposed that the demand for HSA is expected to supersede 1000 metric tons by the year 2020. The African and Asian markets show the most potential for the sale of plasma derived products. The Asian HSA markets are expected to grow at greater than 7% per year whilst Africa represents a unique, untapped market for plasma derived products.

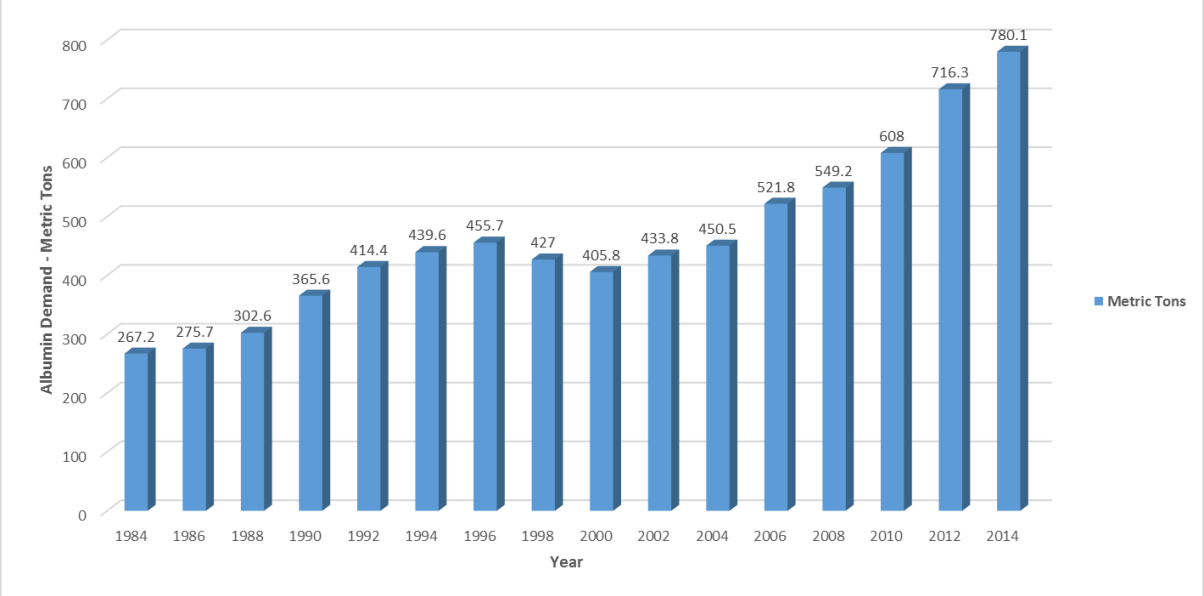


Figure 2:2: *Graphical representation of the worldwide global demand for human serum albumin (metric tons/year) from 1984 to 2014. The trend shows a year on year increase in demand for albumin, with a global drop in demand from 1998 to 2000. Increase in demand of albumin is expected to continue through to 2002, with more than 1000 metric tons expected to be used worldwide in this year (Robert, 2014).*

In the plasma fractionation industry, the cost of the raw material (i.e. human plasma) is extremely high, when compared to the raw materials of other biopharmaceutical industries. The Department of Transfusion Medicine in Italy undertook a recent study to determine the cost of 1L of plasma, collected via. several methods including whole blood and apheresis. In this study, Eandi *et al.*, (2015) calculated the cost of 1L of plasma from collection to delivery at fractionation centres, including donor testing

costs, plasma testing cost, material and transport costs, is approximately €113, for plasma recovered from whole blood, and €276, for plasma recovered via apheresis methods. The cost of manufacturing PDMP's is further increased by other factors including (Burnouf, 2012):

- The economics of producing PDMP's requires that several products be produced from 1L of human plasma, to make the fractionation process cost effective and profitable. This leads to a complex manufacturing processes to extract several plasma proteins at the required yields and purity levels to have significant therapeutic values.
- Plasma fractionation facilities must conform to GMP and stringent design standards to ensure safety, efficacy and quality of products manufactured.
- The advent of viral reduction strategies and implementation during fractionation processes leads to further increases in manufacturing costs.

The projected demand for HSA is expected to increase year on year with at least 1000 metric tons of HSA required by the year 2020. The African and Asian markets show the most potential for growth with respect to the sale of PDMP's, especially HSA. The production cost of HSA is increased by the cost of recovery and testing of human plasma, which is the raw material used manufacture plasma derived products. The cost of human plasma in South Africa varies, therefore, the costs of this raw material is illustrated by using a recent study conducted by Eandi *et al.,* 2015 who estimated the cost of plasma at €113/L (approximately R1700/L; exchange rate R15.11).

2.2.3 THE HISTORY OF PLASMA FRACTIONATION

Plasma fractionation methods were developed in the early 1940's in response to the requirement for human albumin and immunoglobulin during World War II (Burnouf, 2012; Curling and Bryant, 2005). In 1946 Edward J Cohn, a world renowned protein biochemist, and his colleagues proposed a method for the separation and purification of protein components from human plasma (Cohn *et al.*, 1946). The Cohn fractionation method is based on exploiting the solubility characteristics of proteins by controlling ionic strength, pH, temperature, protein concentration and organic precipitant (ETOH) concentration (Cohn *et al.,* 1946). The methods proposed by Cohn *et al.*, (1946) are

recognised as the cornerstone process strategy for fractionation of human plasma (Burnouf, 2012).

Several scientists contributed significantly to improving the process schemes described by Cohn *et. al*., (1946). In 1962, Kistler and Nitschmann described a method for the large scale fractionation of plasma proteins from human plasma (Kistler and Nitschmann, 1962). The Kistler and Nitschmann (1962) method is largely based on the Cohn cold ETOH fractionation scheme and is optimized for process time, improved product yields and purity whilst using reduced volumes of reagents and materials. The purity of albumin extracted by the Kistler and Nitschmann (1962) method is 2-3% purer than that extracted by other methods, whilst the yield of albumin remains unchanged. The purity of immunoglobulin extracted by the Kistler and Nitschmann (1962) method is approximately 99% (similar to alternate methods). However the yield of immunoglobulin is more than 1% higher in the Kistler and Nitschmann (1962) method. A further key benefit of the Kistler and Nitschmann (1962) method is that the fractionation process is specifically optimized for the recovery of Fraction V paste and Fraction II paste which contains HSA and immunoglobulin respectively, through separate process steps. Also, Kistler and Nitschmann (1962) method of fractionation is preferred since it has the added advantage of virus removal through partitioning of viruses during the various solid-liquid separation steps conducted during the process. However, cold ETOH fractionation is also supported by other viral inactivation steps e.g. inactivation at elevated temperatures to ensure complete removal of viruses (Dichtelmüller *et al.,* 2011).

An alternate method to the Cohn Cold Ethanol Fractionation scheme is the chromatographic plasma separation technique, devised by Curling *et al*., in the 1970's. In this process plasma proteins are separated based on their size and charge and not on their solubility characteristics. Today, most organizations have developed their own methods of fractionation to improve productivity, yield and purity. Many fractionation methods are based on those described by Cohn *et al*., (1946), Kistler and Nitschmann (1962) or Curling *et al.,* (1977); it is not uncommon for organizations to use a combination of all three methods. The fractionation processes utilized at NBI are based on the Kistler and Nitschmann method of 1962.

2.2.4 PLASMA FRACTIONATION AT NBI

The cold ethanol fractionation scheme, based on the methods described by Kistler and Nitschmann (1962), to manufacture various plasma proteins on an industrial scale is shown in Figure 2:3. In this process, plasma proteins such as albumin and immunoglobulin are precipitated from human plasma by specifically exploiting the solubility characteristics of proteins, which depend on ionic strength, pH, temperature, protein concentration and changes in solvent polarity which is influenced by organic precipitant concentration e.g. ETOH concentration. Plasma proteins of interest are then recovered by using various solid-liquid separation techniques such as centrifugation or depth filtration. The intermediate protein precipitates/paste, referred to as Fractions, are reconstituted and subjected to purification processes (which include dissolution of the Fractions, depth filtration of the dissolved Fraction, to remove larger particles and finally polishing of the protein solution through UF) prior to formulation and final filling into product containers.

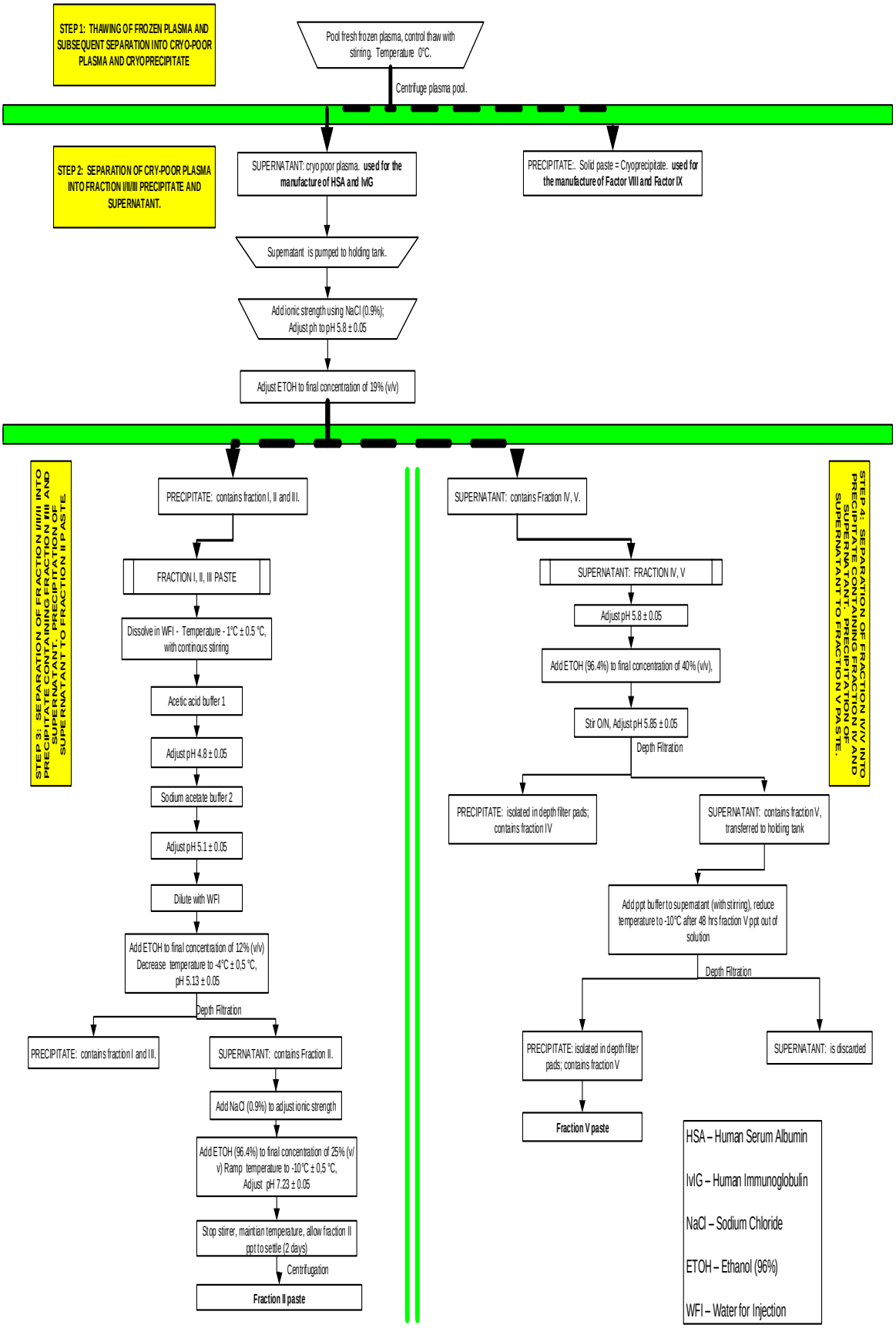


Figure 2:3: *Process flow diagram showing the fractionation of Fraction V paste (containing albumin) and Fraction II paste according to the methods of Kistler and Nitschmann (1962); as practised by the National Bioproducts Institute*

The methods of Kistler and Nitschmann (1962) adopted by NBI to manufacture HSA can be divided into two unique stages. In the first stage, referred to as the extraction of Fraction V paste from human plasma pools (Section 2.2.4.1), the protein of interest is separated from other plasma proteins by exploiting their specific solubility characteristics, and thereafter using solid-liquids separation techniques to separate proteins. During this stage, HSA is produced in the form of a paste, referred to as Fraction V paste. Also, immunoglobulin are separated as a paste and referred to as Fraction II paste. In stage two of the fractionation process, referred to as the purification of Fraction V paste (Section 2.2.4.2), this paste containing HSA is dissolved in a WFI-ETOH admixture and polished to remove all impurities, solvents and salts by UF. The optimization of the ultrafiltration of dissolved Fraction V paste containing HSA is the core focus of this study. The Fraction II paste containing immunoglobulin is also refined using similar methods, but these will not form part of this study. In the following sections, the two unique stages of the Kistler and Nitschmann (1962) method, as practised by NBI, will be discussed in more detail.

2.2.4.1 EXTRACTION OF FRACTION V PASTE FROM HUMAN PLASMA POOLS

Initially, Fraction V paste containing HSA is separated from pools of human plasma (Figure 2:3). The process can be separated in various steps and is described in detail as follows:

- In step one of this process, frozen human blood plasma, generally in plastic packs of approximately 250 - 400mL volume are pooled and control thawed in temperature controlled vessels. The thawed plasma is subjected to centrifugation and the precipitate (also known as cryoprecipitate) contains fibrinogen and other clotting factors (approximately 50% concentration), which is used for the manufacture of Factor VIII and Factor IX. The supernatant (also known as cryo-poor plasma) contains various serum proteins including albumin and immunoglobulin and is transferred to a holding tank.
- In step two, ETOH, to a final volume of 19% (v/v) is added to the cryo-poor plasma (supernatant from the preceding step) and the pH is adjusted to 5.8 ± 0.02, the solution is subjected to centrifugation. The precipitate recovered after centrifugation contains immunoglobulin (approximately 80% concentration) and is known as Fraction I/II/III. The supernatant recovered

after centrifugation contains albumin (approximately 80% concentration) and is referred to as Fraction IV/V.

- In step three, Fraction I/II/III is reconstituted in WFI, at low temperatures (1°C ± 0.5°C). Acetic acid and sodium carbonate buffers are added to the reconstituted Fraction I/II/III solution, to maintain pH (5.13 ± 0.05) and ionic strength (0.0014). Thereafter, ETOH (12%, v/v) is added to the solution. This solution is subjected to depth filtration, the precipitate (containing 90% α and β globulins, 7% clotting factors and 2% other proteins) is known as Fraction I/III and is discarded. The supernatant (containing 92% immunoglobulin and 8% albumin) is retained for further processing. The ionic strength of this supernatant is increased (from 0.014 to 0.04) using buffer, pH adjusted to 7.3 ± 0.05 and temperature lowered to -10°C ± 0.5°C. These process conditions facilitate the precipitation of Fraction II paste which contain approximately 95% immunoglobulin, which is recovered by centrifugation. Fraction II paste is reconstituted and refined prior to formulation and filling into product containers.
- During step four, ETOH of 40% (v/v) is added to Fraction IV/V, thereafter, the pH is adjusted to 5.8 ± 0.05. This solution is subjected to depth filtration, the recovered precipitate known as Fraction IV is discarded since it contains transferrin (80% concentration), immunoglobulin (10% concentration) and little albumin (6% concentration). The supernatant is transferred to a holding tank, acetate buffer is added and the temperature is decreased to -10°C ± 0.5°C. These process conditions cause the precipitation of albumin into a paste form known as Fraction V. This Fraction V paste is separated from the bulk liquid by depth filtration. Fraction V paste is reconstituted and refined prior to formulation and filling into product containers. As mentioned previously, this process is the focus of this study and as such will be discussed in more detail in subsequent sections.

2.2.4.2 PURIFICATION (REFINING AND POLISHING) OF FRACTION V PASTE CONTAINING HSA

Fraction V paste, containing approximately 28% albumin is recovered as a paste (by depth filtration), after step four of the fractionation process, described in section 2.2.4.1 and Figure 2:3. Fraction V paste is weighed, bagged and stored in a freezer, set at temperatures below -20°C. This API (Fraction V paste) will be dissolved and purified to produce HSA, suitable for parenteral administration (Figure 2:4). The purification of HSA begins with the dissolution of a certain mass (currently 195kg) of Fraction V paste. This paste is dissolved in an admixture of WFI and ETOH (96%, v/v); ETOH final concentration is 10% (v/v). The Fraction V paste is added to this WFI-ETOH admixture when the temperature of the solute is 4°C ± 1°C. The pH is adjusted (using 2M NaOH or 2M HCL) to 4.57 ± 0.05 and the temperature reduced to -3.5°C ± 0.5°C. The solution is stirred overnight and is depth filtered, using a horizontal filter press. Following depth filtration, the precipitate (salts and other proteins) is discarded and the filtrate (containing HSA) is recovered and concentrated (first concentration step) to a protein concentration of approximately 80g/L, while the pH is adjusted to neutral (7.2 ± 0.02). Ethylenediaminetetracetic (EDTA) acid is added to the solution as a chelating agent. The solution is then ultrafiltered by the method of constant volume diafiltration (CVD) using WFI as the diafiltration diluent. The volume of diafiltration diluent used during CVD is equivalent to five times the volume of solution measured at the start of CVD, were each volume of diafiltration diluent constitutes one diafiltration step. Following CVD, the HSA solution is concentrated (second concentration step) to a final protein concentration acceptable for parenteral use, e.g. 20% or 4% protein concentration. This HSA bulk liquid is formulated with stabilizers and then submitted for sterile filling. Following aseptic, sterile filling of HSA into final containers, the product is pasteurized at 60°C for 10 hours.

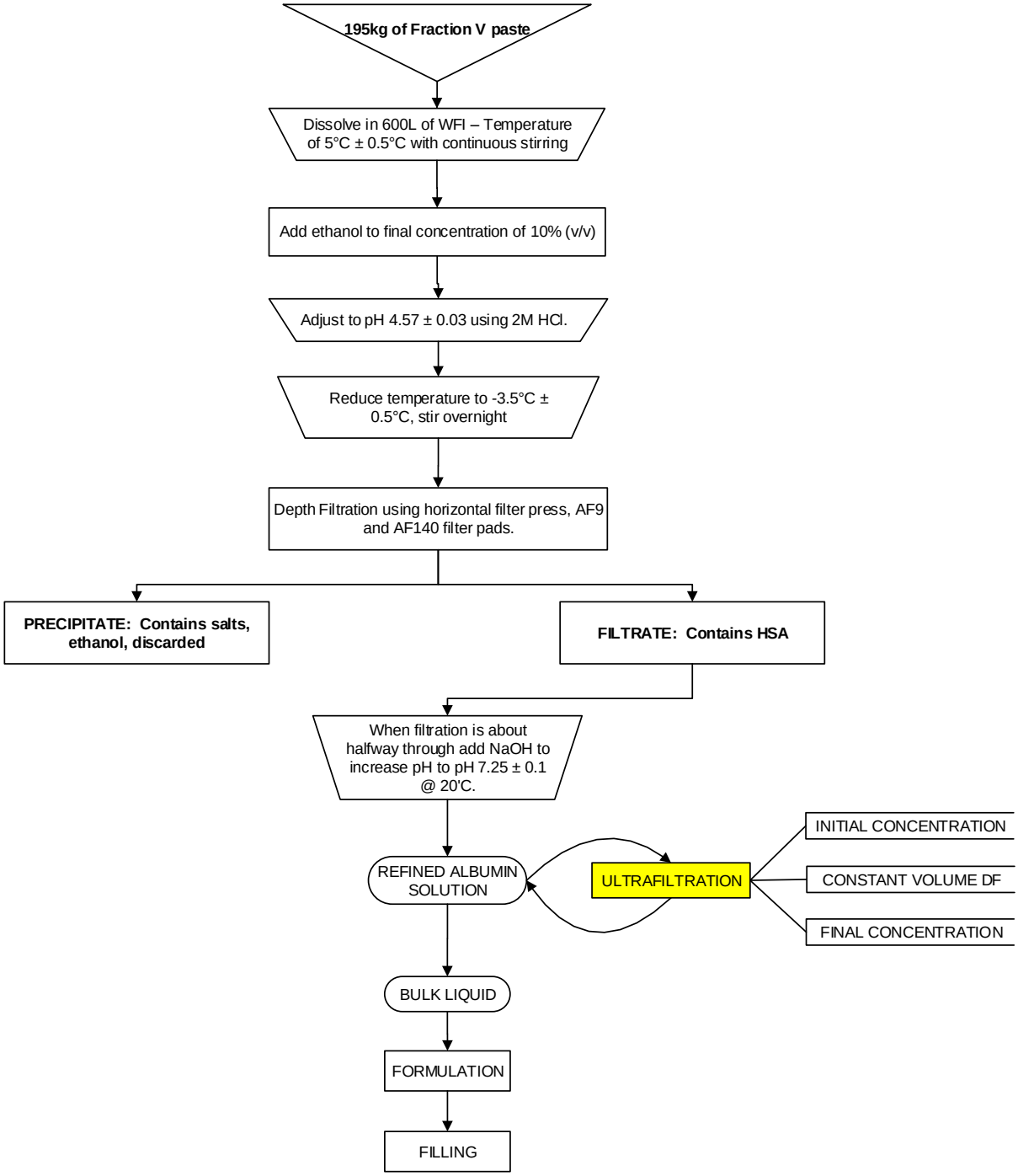


Figure 2:4: *Process flow diagram depicting the dissolution, depth filtration and ultrafiltration of Fraction V paste containing albumin. This diagram also shows the key set points for temperature, pH, and ETOH concentration.*

2.3 HUMAN SERUM ALBUMIN

2.3.1 INTRODUCTION

Human serum albumin (HSA) was one of the first plasma proteins discovered and is greatly valued for its various physiological and therapeutic properties. HSA has been used in various biotechnology applications such as fusion proteins and drug delivery systems (Fanali *et al.,* 2012). The structural topology of HSA confirms its ability to bind a large range of hydrophobic molecules and other materials including metals (Cu^{2+}; Zn^{2+}), amino acids, fatty acids and metabolites (Zunszain *et al.,* 2003; Dockal *et al.,* 1999; Sugio *et al.,* 1999). HSA is the most abundant protein found in human plasma, constituting more than half of the plasma protein content (Bosse *et al.,* 2005; Anderson and Anderson, 2002; Sugio *et al.,* 1999; Putnam, 1975). HSA is a common contaminant in various other preparations of plasma derived products, due to its high concentration in human plasma (Putnam, 1975). This extraordinary ability of HSA to bind compounds, coupled with its exceptional abundance in human blood systems, makes it an ideal molecule for carrier of key blood metabolites and to determine the pharmacokinetics of key drugs (Fanali *et al.,* 2012).

This plasma protein is highly soluble in water and is known for its stability under denaturing conditions, when compared to other plasma proteins. This stability under denaturing conditions is conferred to HSA by strengthening of the structural backbone of the molecule by disulphide bridges (Matejtschuk *et al.,* 2000; Sugio *et al.,* 1999). This structural stability of HSA allows for significant variation and optimization of the manufacturing process used to fractionate this product.

2.3.2 PRIMARY STRUCTURE

Human serum albumin (HSA) is a globular protein made up of a single polypeptide chain containing 585 amino acid residues with 17 disulphide bridges (Fanali *et al.,* 2012; Sugio *et al.,* 1999; Dockal *et al.,* 1999). The 17 disulphide bridges are formed from 34 cysteine amino acid residues and confers structural stability to the protein molecule, such that environmental conditions that may denature other proteins can be used to purify HSA (Matejtschuk *et al.,* 2000). The disulphide bridges are not easily accessible by solvents or reducing agents (Carter and Ho, 1994). The geometry and position of these intra-domain disulphide bonds not only provides structural stability to

the protein, but also clusters aliphatic and aromatic amino acid residues towards the inner zones of the protein molecule thus creating a hydrophobic core (Sugio *et al.*, 1999). Human serum albumin is a helical protein (approximately 67%), showing a typical internal atomic structure of hydrogen bonded loops and several turns (Carter and Ho, 1994). This protein (HSA) is composed of three similar domains, coded I, II, III; each domain is made up of two subdomains coded IA, IB, IIA, IIB, IIIA, IIIB (Dockal *et al.*, 1999). The arrangement of the 3 domains resembles a heart shape structure.

Human serum albumin contains a high concentration of aspartic acid (39 residues per molecule of HSA), glutamic acid (60 residues per molecule of HSA), lysine (58 residues per molecule of HSA) and leucine (61 residues per molecule of HSA) amino acids (Table 2:1). HSA has a net negative charge (-15) at neutral pH. Furthermore, the large number of ionisable amino acids makes HSA an extremely soluble molecule (Fanali *et al.*, 2012).

Table 2:1: *Table showing the amino acid residues of human serum albumin. Glutamic acid (60), Leucine (61) and Lysine (58) are the most common amino acid residues in human serum albumin.*

AMINO ACID	RESIDUES
Asp	39
Asn	15
Thr	30
Ser	22
Glu	60
Gln	23
Pro	25
Gly	12
Ala	63
Cys	35
Val	39
Met	6
Ile	8
Leu	61
Tyr	18
Phe	30
His	16
Lys	58
Trp	1
Arg	23

2.3.3 PHYSICAL AND CHEMICAL PROPERTIES

Human serum albumin (HSA) has a molecular weight of 66438 Daltons (Fanali *et al.*, 2012). The molecule has an isoelectric point of 4.7, which is the lowest of all major plasma proteins (Matejtschuk *et al.*, 2000). Other key physical properties of HSA include (Schultze and Heremans, 1966):

- Intrinsic viscosity of 0.042
- Extinction co-efficient of 5.8g/L at absorbance of 280nm.

HSA in blood plasma has a half-life of approximately 12-16 hours, whilst HSA endogenous to the blood system in human beings has a half-life of approximately 3-4 weeks (Liumbruno *et al.*, 2009).

2.3.4 PHYSIOLOGY AND THERAPEUTIC SIGNIFICANCE

Human serum albumin (HSA) is synthesized in the hepatic cells of the liver at a rate of approximately 200mg of HSA/kg body mass, i.e. approximately 15g of HSA per day in a 70kg man (Hastings and Wolf, 1992; Tulis, 1977). HSA has a typical blood concentration level of approximately 5g/100mL (30-40g/L) (Anderson and Anderson, 2002; Sugio *et al.*, 1999). The primary physiological role of HSA includes transporting of exogenous compounds such as pharmaceutical drugs and endogenous compounds such as bilirubin (Zunszain *et al.*, 2008) and heme (Zunszain *et al.*, 2003). This ability to transport various substances is aided by a high net negative charge and increased solubility that allows efficient binding of various substances (Hastings and Wolf, 1992). Due to its high solubility and high net negative charge, HSA generates the colloid osmotic pressure that regulates the movement of water and diffusible salts through capillaries (Olsen *et al.*, 2004).

The therapeutic uses of albumin include treatment of shock, thermal injuries (e.g. burns), therapeutic plasmapheresis and restoration and maintenance of circulating blood volume (Hastings and Wolf, 1992; Liumbruno *et al.*, 2009). HSA is regarded as a reference point for identifying several disease conditions including cancer, rheumatoid arthritis and ischemia (Fanali *et al.*, 2012).

2.3.5 A MODEL SUBSTITUTE: BOVINE SERUM ALBUMIN

Bovine serum albumin (BSA) is used in many UF experiments as a test protein since it is readily available at low cost and is biochemically very similar to HSA. Albumin isolated from different mammalian species share many similar physic-chemical properties including surface hydrophobicity, isoelectric point and molecular weight (Putnam, 1975; Michnik *et al.*, 2006). Akram *et al.*, (2011) used sequence alignment techniques to evaluate (at the molecular DNA level) the similarity and identity of HSA with albumin derived from other mammalian species (BSA, cat serum albumin, rat serum albumin, pig serum albumin). This study showed that HSA and BSA have a similarity of 88.0%; only cat serum albumin showed a higher similarity (90.6%) to HSA than BSA.

Table 2:2: *Table showing the amino acid residues of bovine serum albumin. Note the strong homogeneity with human serum albumin amino acid content. Glutamic acid (58), Leucine (65) and Lysine (60) are the most common amino acid residues in bovine serum albumin.*

AMINO ACID	RESIDUES
Asp	41
Asn	14
Thr	34
Ser	32
Glu	58
Gln	21
Pro	28
Gly	17
Ala	48
Cys	35
Val	38
Met	5
Ile	15
Leu	65
Tyr	21
Phe	30
His	16
Lys	60
Trp	3
Arg	26

The minor differences between HSA and BSA lies in the number (HSA has 585 amino acids whilst BSA has 582) and composition of amino acid residues (Table 2:2). Also, BSA has a net negative charge of -17 whilst HSA has a net negative charge of -15.

3 ULTRAFILTRATION: A KEY MEMBRANE TECHNOLOGY AND ITS APPLICATION IN PLASMA FRACTIONATION

3.1 INTRODUCTION TO MEMBRANE TECHNOLOGY

Membrane technologies are used extensively in various industries including pharmaceutical, biotechnology, waste water treatment, textile, dairy and beverage industries (Vincent-Vela *et al.*, 2012). Membrane technologies include reverse osmosis (RO), UF, nanofiltration (NF) and microfiltration and can be generally described as the pressure driven separation of liquid solutions across a medium (e.g. porous membranes) into separate components, where separation is primarily based on the size of the molecule being recovered (Peeva *et al.*, 2012; Bowen and Williams, 1996). RO has the propensity to remove inorganic contaminants, ions, endoxtoxins and pyrogens due to its very small pore size (approximately 0.001µm). RO requires significant capital investment costs and pre-treatment of feed solutions, but with the main advantage of removing low molecular constituents (< 20000Da). RO is generally used in the waste water industry and in the manufacture of fruit juice concentrate. NF is used mainly to remove divalent ions and molecules in the range 1000 – 100000 Da. NF is generally used in de-salting processes such as production of lactose from cheese whey. The membrane process of UF is primarily based on size exclusion, i.e. molecules with a molecular weight lower than the molecular weight cut off (MWCO) rating of the membrane will pass through the membrane, whilst those molecules higher than the MWCO rating will be retained. UF is used in the diafiltration and concentration of various proteins, purification of recombinant drugs and enzymes (Lim and Mohammad, 2010). MF is used as a pre-treatment in waste water applications and other purification processes that requires separation of large molecules. Microfiltration is characterized by membranes with large pores (>1000000 Da).

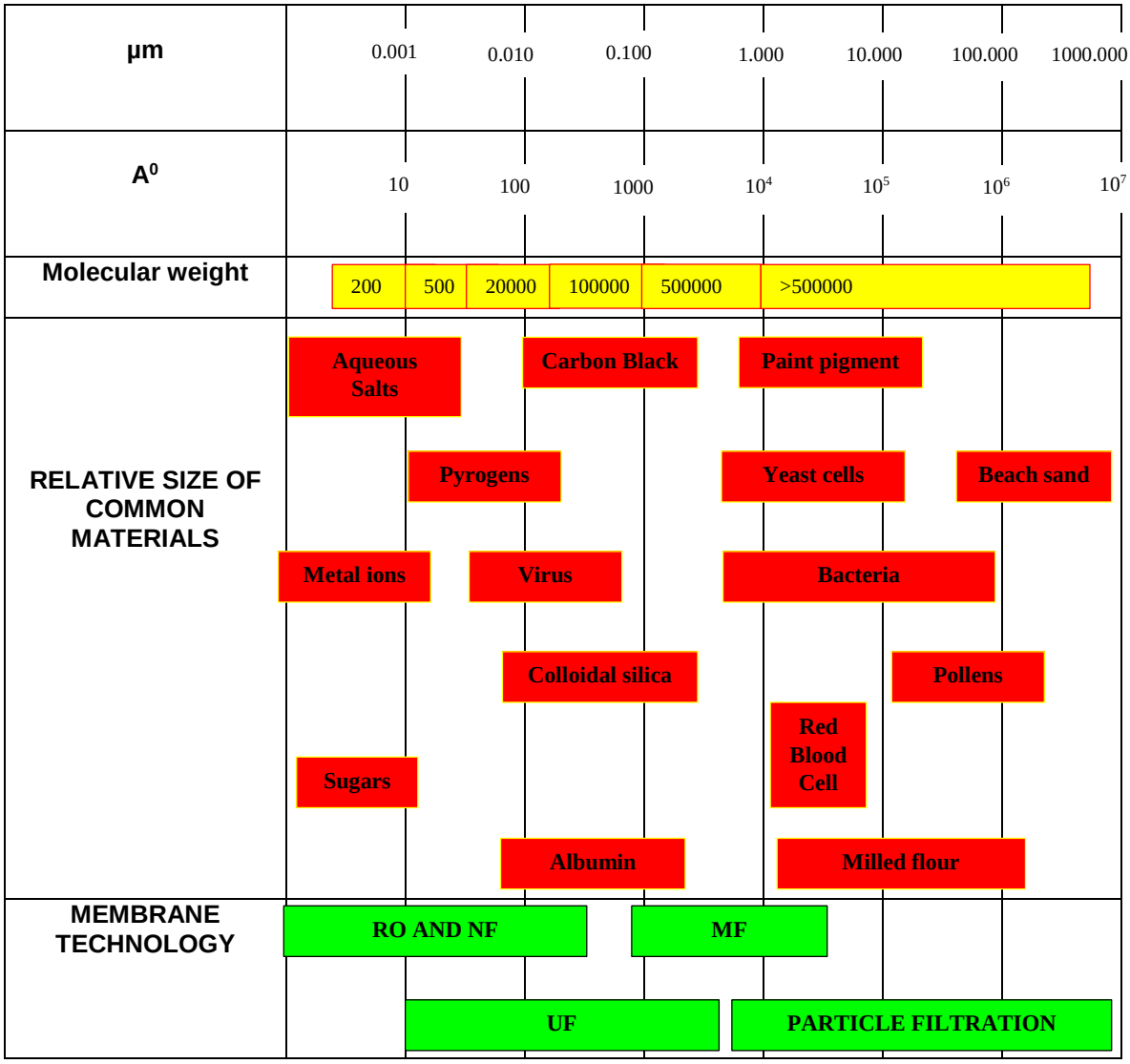


Figure 3:1: *Figure showing the size spectrum of various membrane technologies. Ultrafiltration is used to remove particles of approximately 500 to 100000 Dalton's (Sustainable Sanitation and Waste Management, 2015).*

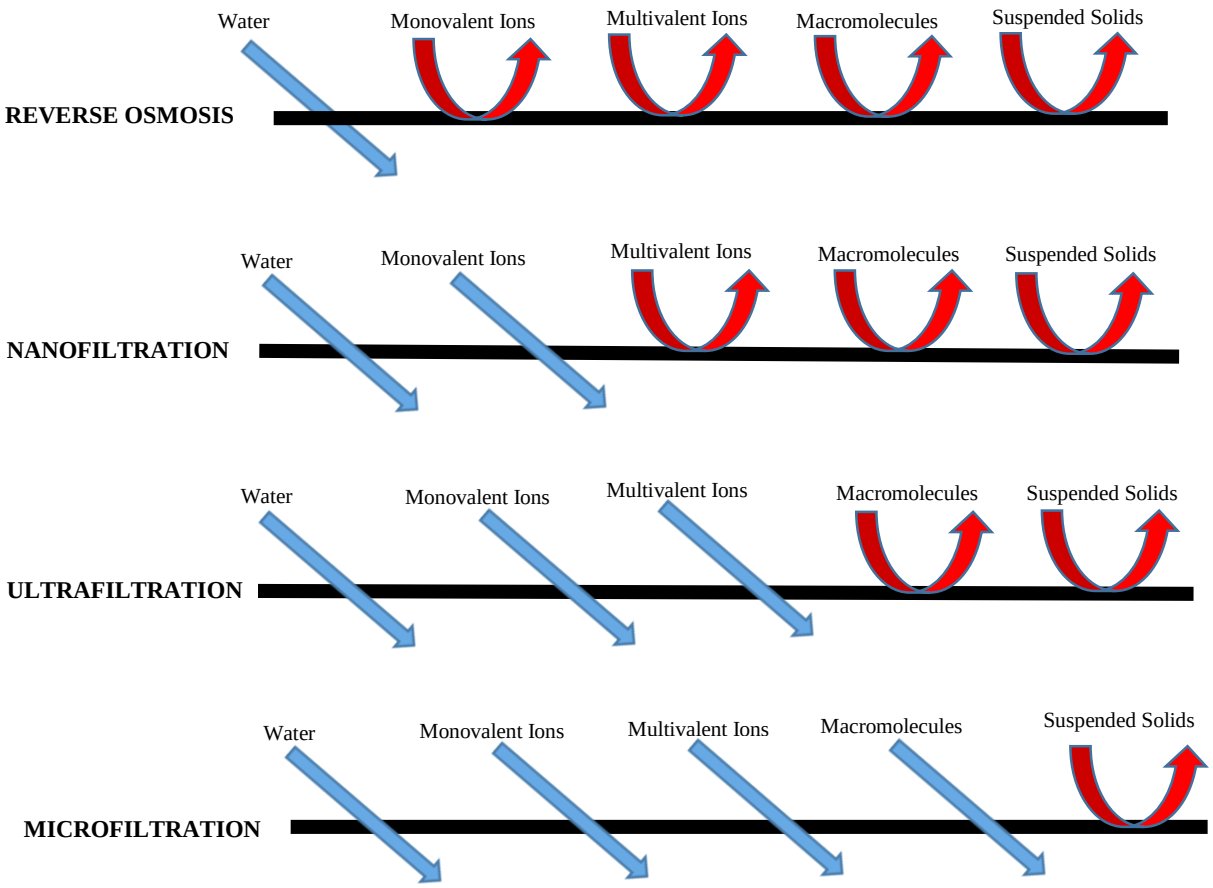


Figure 3:2: *Figure shows the rejection profile for various membrane technologies. During ultrafiltration solvents such as water and different ions pass through the membrane whilst macromolecules e.g. albumin and suspended solids are retained.*

Membrane technologies are generally used as downstream separation, purification or clarification steps or as part of a sequence of separation processes, e.g. purification or refining of human serum proteins (Shao and Zydney, 2003). Membrane technologies have numerous advantages when compared to other methods of purification. The most significant advantages of membrane technologies are (Shirazi *et al.*, 2010):

- High yield recovery of the product of interest
- Separation of components can be achieved under mild conditions
- Reduced operating cost
- Less labour intensive
- Improved automation of process

- Scale up from laboratory experiments to pilot scale and manufacturing scale is easily achieved

The key disadvantages of membrane technologies include (Shirazi *et al.*, 2010):

- Concentration polarization
- Membrane fouling
- Reduced membrane life due to negative impact of CP and MF
- Cost of consumables;

The membranes used during UF of proteins act as selective barriers since they contain pores of a defined size that exclude components larger than the pore size. The size rating of the pores, represented as the MWCO value, depends on the size of molecules present in the liquid and the size exclusion criteria or selectivity of the UF process. There are two modes of UF of proteins that are universally recognized (Figure 3:3) namely normal flow filtration (also known as dead end filtration) and tangential flow filtration (also known as cross flow filtration). These modes of UF are distinguished on the basis of liquid flow as follows (Millipore Application Note, 2003):

- Normal Flow Filtration (NFF) – in NFF, the liquid or feed stream is driven perpendicularly towards the membrane. This technique aims to pass 100% of the liquid through the membrane.
- Tangential Flow Filtration (TFF) – in TFF, the liquid or feed stream is driven parallel to the surface of the membrane. In this technique, a portion of the liquid passes through the membrane and the other portion is recirculated back to the feed reservoir.

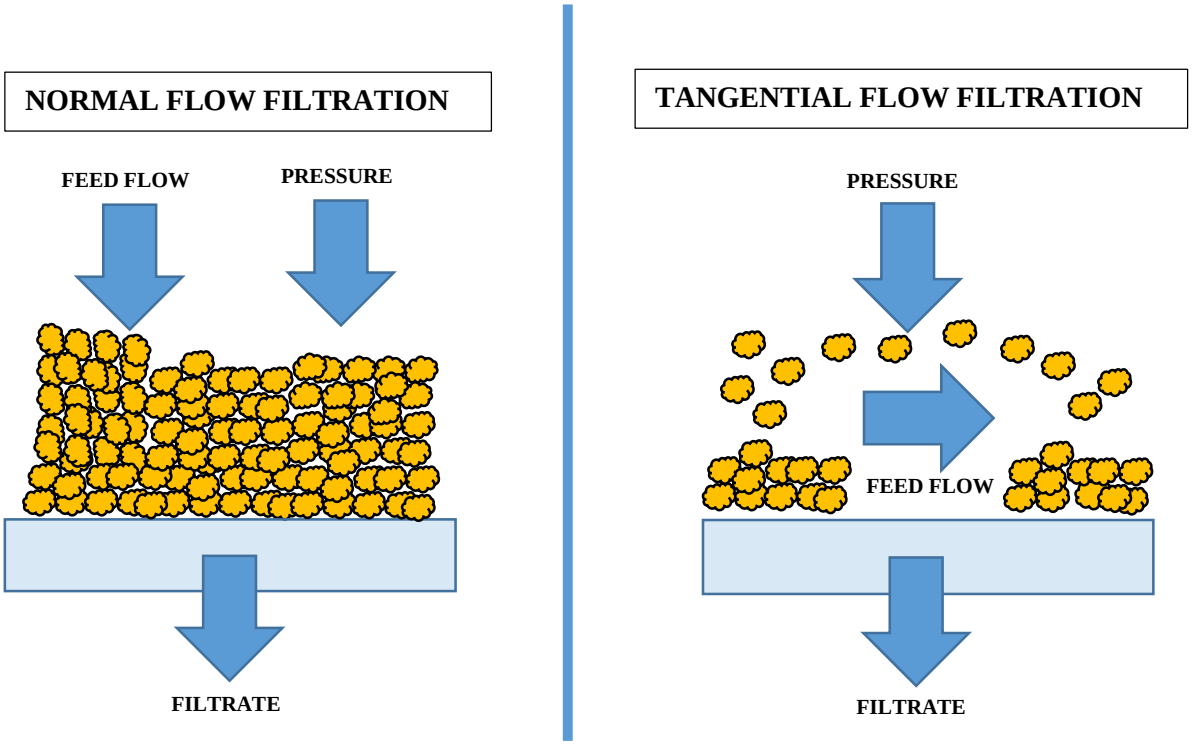


Figure 3:3: *Figure showing the difference between normal flow filtration and tangential flow filtration. In normal flow filtration the pressure driving force and the direction of the feed flow are in the same direction. In tangential flow filtration the direction of the pressure driving force and the feed flow are perpendicular to each other (Millipore Application Note, 2003).*

3.2 MEMBRANES IN MEMBRANE TECHNOLOGIES

The ability of membranes to separate a diverse range of molecules or components has been well known since the 1800's; the fundamental theories of membranes and membrane separations were in part established during these initial years culminating in the first key applications of membranes in separation processes where they were used in World War II for the examination of water (Cuperus and Smolders, 1991). Membranes can be broadly described as a selective barrier between two separate phases and thus regulates the transport of components between the two phases; where this selective transport of components is based on both the physic-chemical properties of the membrane and the feed solution (Shirazi *et al.*, 2010; Saxena *et al.*, 2009; Ulbricht, 2006). The key advantage of membranes is the efficient size exclusion separation principle with defined uses in microfiltraiton, UF, NF and RO applications. This size exclusion principle is characterized by the porous, anisotropic nature of membranes which are manufactured from several polymeric and inorganic materials (Cuperus and

Smolders, 1991). Most recently, researchers have explored the potential of charged membranes to separate charged proteins/biomolecules. These high performance tangential flow filtration (HPTFF) techniques are an emerging membrane separation technology that is gaining in popularity due to its ability to minimize CP and MF (Saxena *et al.*, 2009). Membranes are particularly useful in the pharmaceutical industry; therefore, membrane manufacturers are committed to developing membranes that efficiently remove viruses whilst maintaining high protein separation efficiency (Charcosset, 2006). Further, membranes are designed to minimize MF i.e. have excellent anti-fouling properties which include high retention of the protein of interest (rejection co-efficient), low permeate flux decline, low filtration resistance and low surface roughness (Corbatón-Báguena *et al.*, 2015).

3.2.1 CHARACTERIZATION OF MEMBRANES

Membranes are generally classified into several broad classes based on their material of construction (organic polymers, inorganic materials); cross section type (isotropic, anisotropic, thin-layer, multilayer); membrane shape/configuration (flat sheets, hollow fibres, capsules) and method of preparation (Ulbricht, 2006). Polymeric, anisotropic, flat sheet polyethersulfone (PES) membranes are commonly used in UF processes, especially in the UF of proteins such as HSA. PES membranes have a high degree of thermal stability, chemical stability, increased mechanical strength with high selectivity and permeability (Zhao *et al.*, 2013; Ubricht, 2006). PES membranes are inert, hydrophobic membranes with a net negative charge; it is these factors that assist in reducing MF during UF of proteins (Zhao *et al.*, 2013).

Membrane performance is defined by the unique characteristics of that membrane whose selection is primarily based on its relevant application in a particular process. Characterization of membranes can be divided into two categories namely performance related parameters (separation efficiency, protein retention, permeability, hydrophobicity and diffusion co-efficient) and morphology related parameters which include surface roughness, porosity, pore size and pore distribution (Cuperus and Smolders, 1991). Porous membranes are defined as membranes that separate macrosolutes and microsolutes based on particle size and sieving. Non-porous membranes separate macrosolute and microsolutes based on the solubility

characteristics and diffusivity of solvents and the related interactions with the membrane (Shirazi *et al.*, 2010). The separation efficiency/selectivity or protein retention of a membrane is defined by its molecular weight cut off (MWCO) where MWCO is broadly defined as the lowest molecular weight of a solute that is rejected by the membrane with an efficiency of ≥90% (Mehta and Zydney, 2005; Cuperus and Smolders, 1991). This MWCO value is directly related to pore size and the rejection efficiency value of ≥90% is not a defined scientific standard but rather an industry based norm for evaluating different membranes (Mehta and Zydney, 2005). Pall Life Sciences User Guide for Membrane Cassette Care and Use Procedures, R00640 Rev B recommends that the MWCO of the membrane be at least 3-6 times smaller than that of the solute being retained. In this study the solute or protein of interest is HSA with a molecular weight of approximately 66kDa, therefore, the membrane selected for these experiments had a MWCO of 10kDa. A PES membrane of 10kDa is currently in use for UF of HSA in the current manufacturing process. The membrane has shown excellent retention of HSA, good permeate flux during UF and good membrane flux recovery after UF.

3.2.2 MEMBRANE MATERIALS AND CONFIGURATIONS/MODULES

Membranes are constructed from organic polymers (PES, cellulose, polyimides) and inorganic materials (metals, oxides and carbon) (Verweij, 2012; Ulbricht, 2006). Inorganic membranes are more complex in structure when compared to organic polymeric membranes and are typically used for the separation of gases e.g. separation of hydrogen (H_2) from coal-derived gas (Verweij, 2012). The key advantages of inorganic membranes include increased chemical, thermal and mechanical stability when compared to polymeric membranes (Shirazi *et al.*, 2010). Polymeric membranes are commonly used in UF applications, especially PES; which is a common material used to manufacture membranes since it has well defined physic-chemical characteristics and is readily available in unlimited quantities (Saxena *et al.*, 2009).

Traditional membrane configurations include flat sheet (plate and frame), spiral wound, and tubular and hollow fibre configurations. The flat sheet or plate and frame configuration resembles a depth filter assembly with membranes arranged in stacks. These configurations are typically used in microfiltration and UF applications, especially in the use of high viscosity feed solutions such as those used in the

fermentation industry (Lipnizki, 2008). The spiral wound configuration is a high surface area configuration consisting of two or more sheets of membrane that are wound around a porous support layer creating a free space for permeate flow and a central space for retentate flow. Spiral wound membrane configurations are very economical membranes since they give a very good price per area membrane ratio. However, these membrane types are difficult to clean and have irregular cross flow patterns (Lipnizki, 2008). The tubular and hollow fibre configurations are similar in structure and are made up of membrane material that is cast on tubes which are connected to end plates. Tubular configuration membranes are generally used with high viscosity feed solutions, but has low packing densities (increased porosity) and requires large energy consumption (Lipnizki, 2008). New membrane configurations are continuously being developed to decrease MF and CP during membrane separation processes (Charcosset, 2006). These new membrane configurations, described as dynamic filtration modules, use vibrating or shaking membranes (pulsating); or rotating disks to destabilize the gel boundary layer that is formed during membrane separation processes (Jaffrin, 2012). Pulsed UF, although not investigated in this study will be discussed in the later part of this literature review (Section 3.4.5).

3.2.3 MEMBRANE CLEANING STRATEGIES

Cleaning of membranes used in membrane separation technologies are critical for maintaining membrane efficiency (hydraulic permeability, permeate flux and protein retention) and overcoming the effects of CP and MF, which is an inherent characteristic of membrane technologies (Levitsky *et al.*, 2012). The membrane cleaning strategy has a direct impact on the life time or ageing of the membrane (Kuzmenko *et al.*, 2005). Ageing is generally defined as changes in physic-chemical structure of the membrane and includes changes in pore size, pore geometry, surface roughness, density, and change in hydrogen patterns (Donose *et al.*, 2013). The effect of a defined cleaning procedure for a particular process is limited by the process conditions that influence MF e.g. protein concentration, TMP, cross flow velocity (CFV), temperature (Diagne *et al.*, 2013). All cleaning strategies will involve the following steps (Levitsky *et al.*, 2012; Corbatón-Báguena *et al.*, 2014):

- An initial rinse of the membrane, after fouling, generally using pure water

- Thereafter, the membrane will be rinsed and soaked in a cleaning solution of relevant concentration. Generally sodium hydroxide or sodium hypochlorite is used but most recently cocktail cleaning solutions (combination of two or more cleaning agents) have been used
- Final rinse of the membrane with pure water to remove the cleaning solution.
- Membrane is stored in a storage buffer e.g. 0.5M NaOH till required for further use.

Chemical cleaning of membranes occurs through chemical interactions between the chemical cleaning reagent, proteins and membrane. The chemical cleaning is dependent on the concentration of the cleaning reagent and the amount of time the membranes are exposed to this reagent (Kuzmenko *et al.*, 2005). In an effort to determine the cleaning efficiency of various chemical reagents including sodium hydroxide (NaOH), sodium hypochlorite (NaOCl) and hydrogen peroxide (H_2O_2); Kuzmenko *et al.*, (2005) fouled PES membranes, of 20kDa, with BSA (0.3g/L) using fixed hydrodynamic and environmental conditions. Thereafter, membranes were cleaned with pure water, NaOH (100, 500, 1000, 3000ppm), NaOCl (100, 1000, 3000, 5000ppm) and H_2O_2 (100, 1000, 3000ppm). The flux recovery, measured after completing chemical cleaning with the mentioned reagents revealed that pure water is ineffective when cleaning membranes fouled by proteins and serves only to remove loosely adhered proteins. NaOH cleaned membranes were only able to restore the permeate flux to 70% of the original permeate flux. NaOCl was the most efficient cleaning reagent, achieving a permeate flux recovery of 95%. Although NaOCl was the most efficient cleaning reagent in this study, the concentration at which the reagent was used is critical to the degree of cleaning. The experimental results showed that as NaOCl concentration increased, the propensity for fouling also increased, after each use of NaOCl as a cleaning reagent. The effectiveness of NaOCl as a cleaning agent is based on its ability to denature proteins thus causing protein aggregates to be removed from the membrane surface and within pores, thereby improving hydraulic permeability of the membrane.

In a study conducted by Carbatón-Báguena *et al.*, (2014), the relationship between hydraulic cleaning efficiency (HCE - %), salt solutions (NaCl – 0mM to 12.5mM), temperature (25°C; 37.5°C and 50°C) and CFV (1.2m/s to 4.2m/s) was evaluated for different membranes (PES, hydrophilic PES and ceramic) with different MWCO (5kDa, 15kDa and 30kDa). The HCE (%) was analysed by first fouling the membrane with BSA (1%, w/w) and $CaCl_2$ (0.06%, w/w); thereafter rinsing with deionized water, cleaning and then a second rinse with deionized water. The high CFV (3.19m/s to 4.2m/s) with fixed NaCl concentration and temperature achieved the highest HCE, for all membranes tested. High CFV induces high shear forces that removes protein molecules from the surface of the membrane. High temperature (50°C) also produced highest HCE at fixed NaCl concentration and CFV. This finding was explained by the increased hydrodynamic flow to the membrane interface resulting in increased interaction of salt molecules with protein. Salt solutions increased HCE with increasing molarity of NaCl, up to a critical concentration beyond which HCE declined. This was true for all membranes tested. This effect was linked to the relationship between salt concentration and surface tension. At low salt concentration, surface tension is low which favours high solubility of the proteins. Carbatón-Báguena *et al.*, (2014) showed that the most effective cleaning procedures for the 5kDa, PES membrane, was achieved with 10mM NaCl, CFV of 3.15m/s at a temperature of 50°C.

The membranes used for the UF of HSA at NBI are cleaned after every use; membranes are flushed with WFI and then cleaned with a low concentration, NaOCl-WFI mix. Thereafter, the cleaning reagent is completely rinsed from the membranes. The membranes are then stored in formaldehyde until required for next use. NaOCl is generally considered a harsh cleaning reagent with the ability to increase ageing of membranes.

3.3 ULTRAFILTRATION: A KEY MEMBRANE SEPARATION TECHNOLOGY

3.3.1 THE KEY STEPS OF ULTRAFILTRATION

The process of UF of proteins is sometimes referred to as traditional diafiltration and can be described by seven key steps based on the addition of diafiltration diluent (or buffer) to the retentate (Paulen *et al.*, 2011; Fikar *et al.*, 2010). These key steps, illustrated in Figure 3:4, are first concentration step, diafiltration step one, diafiltration step two, diafiltration step three, diafiltration step four, diafiltration step five and second concentration step (Wang *et al.*, 2014; Lipnizki *et al.*, 2002). In the first concentration step, the solute to be ultrafiltered (containing macromolecules and micromolecules) is concentrated to an optimum concentration of the macromolecule i.e. the protein of interest, by removal of permeate with no addition of diluent to the retentate thus resulting in a decrease in volume of the feed solution (Fikar *et al.*, 2010). This optimum concentration is based on the propensity for CP and MF and is therefore dependent on key factors that influence these inherent characteristics of UF of proteins.

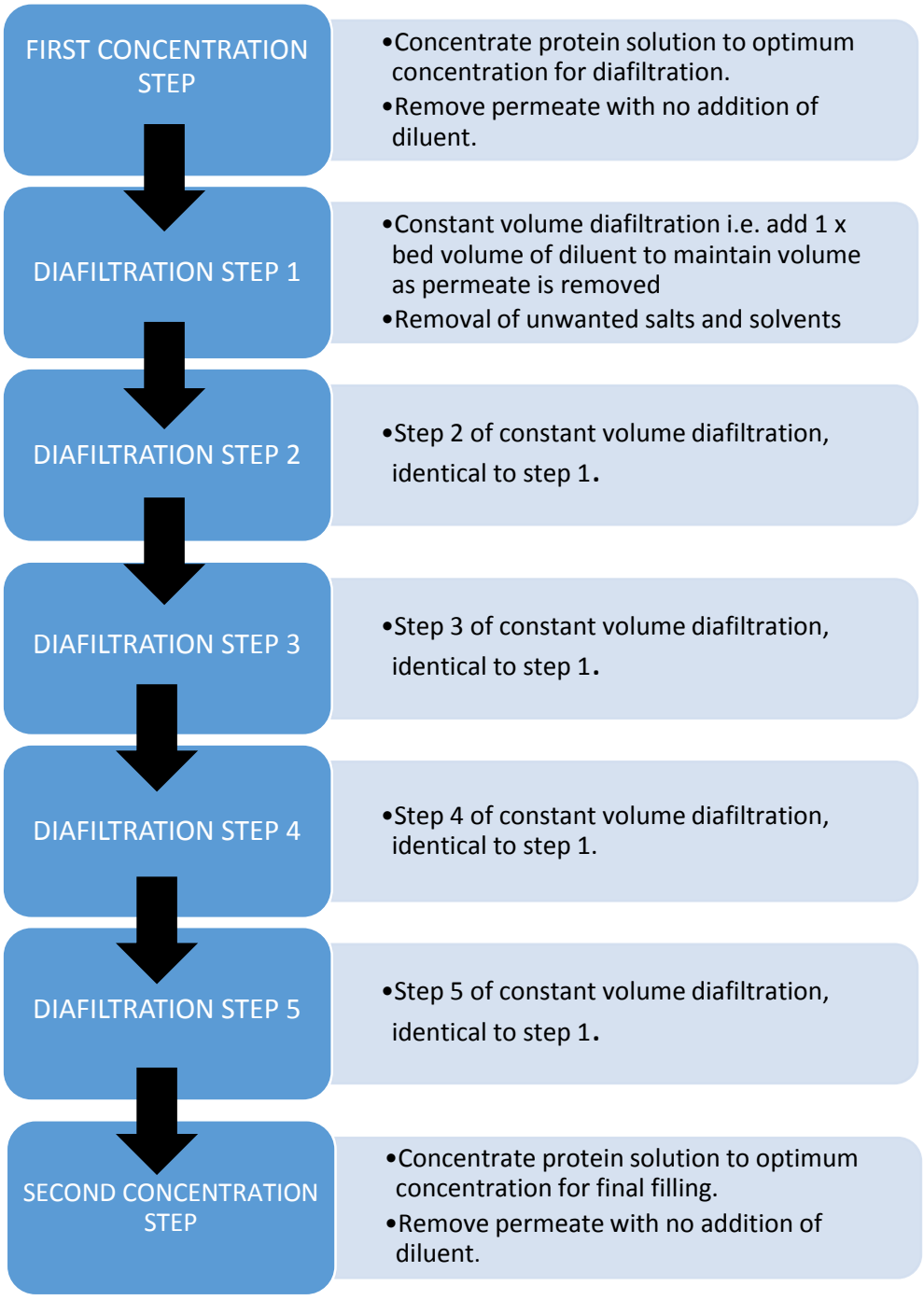


Figure 3:4: *The seven steps of ultrafiltration with respect to proteins are first concentration step, diafiltration steps one to five and second concentration step (Wang et al., 2014).*

The diafiltration steps one to five is characterized by the removal of micromolecules (e.g. sodium) and unwanted solvents (e.g. ETOH) through a "washing" process – by the addition of diluent to the retentate (Figure 3:5). The diluent (e.g. WFI) can be added to the retentate via two methods:

- Continuous feed diafiltration (CFD); also known as continuous volume diafiltration (CVD) – where diluent is added to the retentate at approximately the same rate at which permeate is removed. During CFD the macromolecule concentration remains constant (Foley, 2006).
- Intermittent feed diafiltration (IFD) or variable volume diafiltration (VVD) – where diluent is added to the retentate at rate lower than which permeate is removed such that concentration and diafiltration occur simultaneously. During IFD the macromolecule concentration changes (Fikar *et al.*, 2010; Jaffrin and Charrier, 1994).

In the second concentration step the solute is concentrated to the final concentration recommended for formulation, in a mode similar to the first concentration step i.e. permeate is removed and no diluent is added to the retentate until the required volume is achieved. Figure 3:5 shows the typical protein concentration for each step of the UF of the protein solution and the removal of unwanted solvent such as ETOH, from the protein solution. Protein concentration increases during first concentration step whilst remaining constant during CVD and increasing again in the second concentration step. Ethanol and unwanted micromolecules such as Na follow a similar trend and are removed, in a logarithmic function during UF.

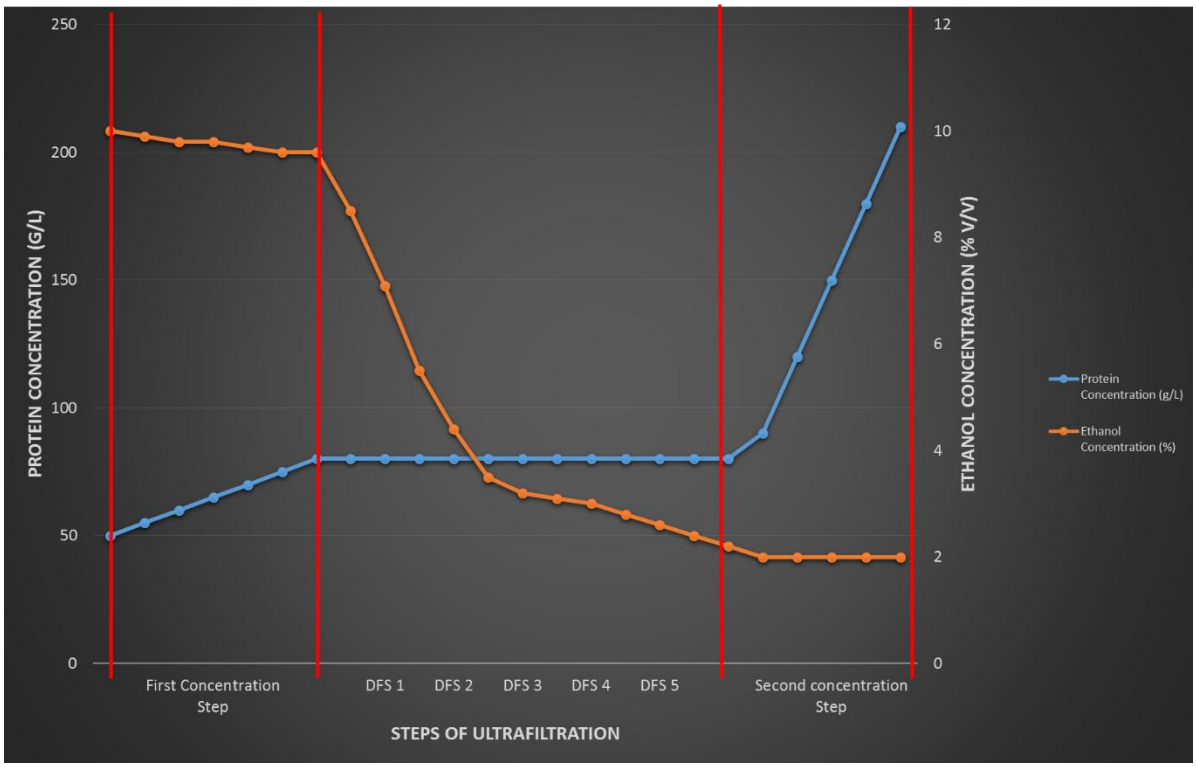


Figure 3:5: *Indicates the seven steps of ultrafiltration and the logarithmic removal of unwanted solvents e.g. ethanol and the protein concentration of each step. In this study, constant volume diafiltration (CVD) method was utilized.*

In a study conducted by Lipnizki *et al.*, (2002), the author's critically analysed three modes of UF of proteins namely batch UF, continuous UF and counter-current UF (Lipnizki *et al.*, 2002). Batch UF and continuous UF of proteins are widely accepted as the traditional methods of completing this process. In batch UF of proteins the three phases of UF described above are completed sequentially, on one membrane stage. In continuous UF of proteins the steps of UF are completed sequentially, but each step is assigned to a different membrane stage. Batch UF requires lower capital expenditure and investment costs when compared to continuous UF. Continuous UF of proteins is applicable to UF of components that have low stability, requiring shorter process times. Generally, pure water is used as the diluent during diafiltration thus the production of pure water adds significantly to the cost of the UF process. Therefore, the counter-current UF system recycles the permeate stream and uses it as diluent during the diafiltration process. The counter-current UF of proteins is applied to different membrane stages (similar to continuous UF), especially where permeate is not required for further processing and generally requires increased membrane surface area.

Following a critical analysis of these UF concepts and using a protein test molecule, Lipnizki *et al.*, (2002) confirmed the following:

- Batch UF requires the least membrane area
- Batch UF consumes the least diafiltration diluent
- Batch UF consumes the least power
- The capital investment costs and membrane costs are the least for batch UF.

The differences between batch and continuous UF of proteins is further highlighted in Table 3:1.

Table 3:1: *Table showing the key differences between batch ultrafiltration process and continuous ultrafiltration process. National Bioproducts Institute utilizes a batch ultrafiltration system. Although the batch ultrafiltration system has a high energy consumption the capital expenditure investment and process control investment are low while allowing high volume throughput (Lipnizki et al., 2002).*

	BATCH	**CONTINOUS**
UF plant investment	Low	High
Batch tank investment	High	Low
Process control investment	Low	High
Space requirements UF	Low	High
Feed volume	High	Low
Protein concentration in permeate	Variable	Constant
Residence time	High	Low
Temperature flexibility	Low	High
Module efficiency	Low	High
Energy consumption	High	Low

NBI completes the UF of HSA according to the three steps described by Wang *et al.*, (2014) and detailed above. During this process the batch UF mode is utilized and WFI is used as the diafiltration diluent during CVD.

3.3.2 ULTRAFILTRATION OPTIMIZATION STRATEGIES

The benefits of process optimization strategies for UF of proteins relate to reducing operational costs whilst producing a high quality product with sufficient yield and purity, through sustainable, energy efficient processes (Paulen *et al.*, 2015; Wang *et al.*, 2014). A further strategic objective for the optimization of UF of proteins, in conjunction with maintaining yields and purity of the macrosolute, lies in the reduction of process time to complete UF whilst using the least volume of diluent during the diafiltration step of UF (Wang *et al.*, 2014; Fikar *et al.*, 2010). Greg Foley (2006) described a method to minimize diluent usage during the diafiltration step by using the VVD method. In this study of Foley (2006), the macrosolute was concentrated to the maximum acceptable concentration, determined by using the gel polarization model (Equation 3:1). Thereafter, the macrosolute was subjected to VVD until the final concentration of the macrosolute was achieved. Although VVD is a unique method of completing diafiltration, the overall benefit with respect to diluent minimization is sometimes conflicting since some experiments reveal reduction in water usage when compared to CVD whilst other experiments reveal results to the contrary of this statement. Further, the economic benefit through the reduction of diluent used is dependent on the type of diafiltration diluent used during diafiltration.

Fikar *et al.*, (2010) outlined the key points for the strategic optimization for UF of proteins:

- Membrane performance, determined by evaluation of rejection of macrosolute and permeate flux must be evaluated for the protein solution
- The optimization goals must be defined i.e. minimize diluent usage or minimize process time
- General cost factors must be considered namely energy costs, diluent costs and costs related to loss of product during UF
- Define all process constraints.

The key objective functions for the optimization strategy related to the UF of HSA, will include the minimization of process time through maximizing permeate flux and maximizing productivity by processing the highest kilograms of protein/hour.

A typical protein concentration, volumetric profile and mass balance for the UF of dissolved Fraction V paste containing HSA, as completed by NBI, is indicated below (Table 3:2).

Table 3:2: *This table shows typical protein concentration, volumetric profile and mass balance for the current ultrafiltration process completed at the National Bioproducts Institute.*

	CURRENT PROCESS	**PROTEIN (HSA) MASS BALANCE (kg)**
Mass of Fraction V paste containing HSA (kg)	195	44
Start batch volume (L)	800	
ETOH concentration at dissolution (%)	10	
Protein concentration after dissolution of Fraction V paste (g/L)	50-60	
Target protein concentration (g/L) after 1st concentration step	80	43
Target volume (L) after 1st concentration step	500-600	
Constant volume diafiltration with WFI equal to 5 x start bed volume of protein solution after 1st concentration step (L)	2500 - 3000	
Protein concentration (g/L) at end diafiltration	80	43
Target protein concentration (g/L) after 2nd concentration step	220	
Target volume (L) after 2nd concentration	170-210	
Final product protein concentration (g/L)	200 or 40	40-42
	PRODUCT RECOVERY (%)	>90

The process above is described in detail in section 2.2.4.2, however, Table 3:2 highlights several key points for optimization of UF of HSA. An increase in productivity for UF of HSA can be achieved by increasing the mass of Fraction V paste dissolved at the start of the process. An increase in the mass of Fraction V paste dissolved, in the current volume of WFI-ETOH (800L) will result in higher than normal protein concentrations, at the start of UF. The negative effect of high protein concentration on permeate flux during UF of proteins is well documented. Generally, the "quick fix" option would be to increase process equipment size to accommodate the increase in batch size such that protein concentration at start of UF is at the current level, resulting in increased capital expenditure and larger "equipment footprints" in the manufacturing facility, which has a negative impact on space requirements. It is therefore important to determine the range of protein concentration that favours the refining of HSA at optimum permeate flux. Increased batch size results in increased volumes of diluent used during diafiltration steps one to five; approximately 2500L to 3500L of WFI is used in the current process. The optimization of UF of proteins must also investigate the possible reduction of the volume of diluent used during this process. The calculated product recovery (>90%) of the current process is acceptable, but greater efficiency is also required in this step.

The NBI carries out the dissolution of Fraction V paste in a WFI-ETOH admixture. The impact of EOTH on proteins, membranes and UF is well documented and will be discussed in following sections. Since other fractionation organizations are known to dissolve Fraction V paste in WFI only, an objective of this study will be to determine the impact of ETOH on the UF of HSA. In considering the impact of ETOH on UF of proteins one must also consider the temperature profile of the depth filtration step of purification of HSA since the freezing point of the dissolved HSA in solution will change if the ETOH concentration is changed from 10% (v/v) and this implies a change in process temperature for the depth filtration step.

The UF of dissolved Fraction V paste uses WFI as a diafiltration diluent to maintain constant volume during diafiltration. Various studies have concluded that an ionic salt solution such as NaCl impacts the flux during the UF of albumin (Swaminathan *et al.,* 1981; Fane *et al.,* 1983; Lim and Mohammad, 2010). This study will evaluate the effect of an ionic solution namely NaCl on permeate flux during UF of HSA.

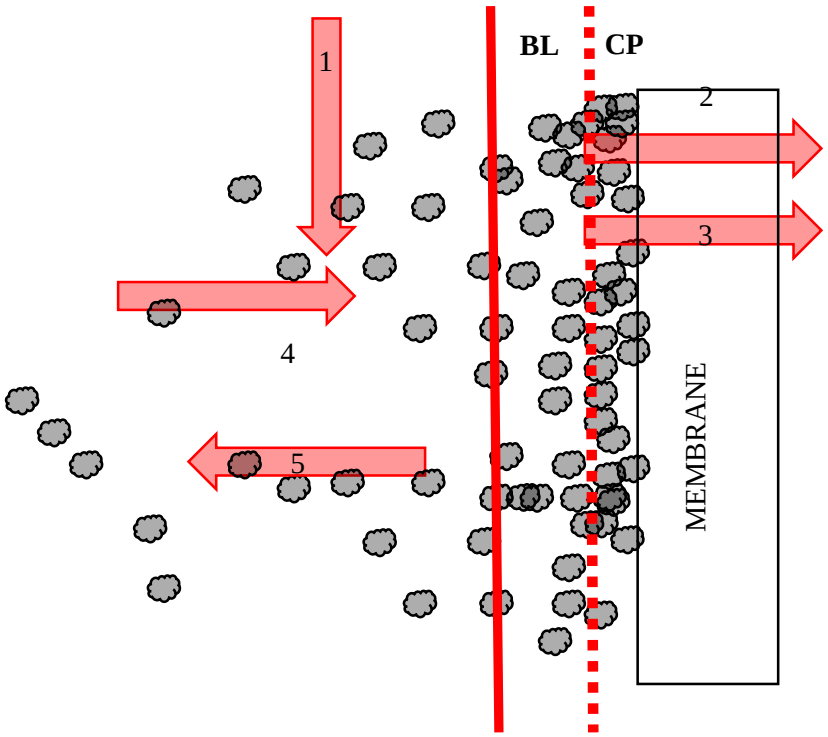
3.3.3 FLUX DECLINE DURING ULTRAFILTRATION MEMBRANE FOULING AND CONCENTRATION POLARIZATION

An inherent characteristic of UF of proteins is the decline in flux during this process. Following extensive investigation of the flux decline during UF of proteins, this phenomenon was attributed primarily to two mechanisms namely CP and MF (Schausberger *et al.*, 2009; Marshall *et al.*, 1993).

In addition to reducing flux, CP and MF increases feed pressure, decreases product quality and reduces membrane life (Shirazi *et al.*, 2010). Thus these mechanisms have a significant impact on process costs, since it increases material costs related to cleaning, energy consumption and membranes (larger membrane surface areas are required to increase flux). Therefore it is important to characterize such fouling mechanisms and through process optimization and equipment design, reduce their net negative impact on the overall process stream.

3.3.3.1 CONCENTRATION POLARIZATION

Concentration polarization (Figure 3:6) can be broadly defined as the time-dependent development of a concentrated layer of solute at the membrane-solution interface, which occurs during UF of a solution e.g. solution containing HSA (Marshall *et al.*, 1993). CP is a function of the hydrodynamic conditions and feed characteristics of the solution being ultrafiltered and is independent of the physical properties of the membrane, i.e. membrane pore size and other physical characteristics of the membrane are not affected by CP (Sablani *et al.*, 2001; Marshall *et al.*, 1993). Concentration polarization is categorized as reversible fouling, since it is not characterized by adsorption of solutes to membrane surface or pore channels and can be controlled by adjusting CFV or pulsation (Sablani *et al.*, 2001).



1 – Feed Flow

2 – Direction of Solute/Retentate Flux

3 – Direction of Permeate Flux

4 – Convective Flow

5 – Back Diffusion

BL – Boundary Layer

CP – Concentration Polarization

.

Figure 3:6: *Diagrammatic representation of concentration polarization phenomenon. Also showing the formation of the boundary layer, back diffusion of solute particles away from the membrane-solute interface, direction of permeate and solute/retentate flux and convective flow of solutes towards the membrane (Sablani et al., 2001).*

The phenomenon of CP has been characterized according to various models, based on the transport mechanisms dominating the UF of protein solutions (Bowen and Williams, 1996). The theoretical aspects of the key models for CP are described in the following sections.

3.3.3.1.1 GEL POLARIZATION MODEL

The gel polarization model or gel layer model is based on the proposition that during UF of proteins and beyond a certain fixed TMP, a gel boundary layer made up of retained solute particles, forms in the vicinity of the membrane surface (Sablani *et al.*, 2001). During UF, these solute particles will continue to accumulate in this gel boundary layer, and therefore the concentration and the thickness of the gel layer boundary layer will increase, until the steady state equilibrium is attained. This steady state equilibrium of solute concentration at the membrane-solute interface is known as the limiting or critical concentration of solute particles on the gel boundary layer. It is observed when the rate of back diffusion (due to convective transport of the solute, driven by TMP) equals the rate of accumulation of solute particles in the gel boundary layer (Shirazi *et al.*, 2010). The hydraulic permeability of the membrane is greatly reduced by the formation of the gel boundary layer of certain thickness (Shirazi *et al.*, 2010; Sablani *et al.*, 2001).

The permeate flux, J, of a solvent limited by gel boundary layer formation can be calculated using the following equation:

$$J = k \ln \frac{C_g}{C_b}$$

J	=	Permeate flux
k	=	Mass transfer co-efficient = $^{D}/_{\delta}$
C_g	=	Concentration of the solute in the gel boundary layer
C_b	=	Concentration of the solute in the bulk solution

Equation 3:1: *Estimate of permeate flux according to the gel concentration model.*

The gel polarization model has several deficiencies in that the theory of this model has not been adequately proven for cross flow filtration due to the assumptions related to mass transfer co-efficient determinations. Further, the concentration of the solute particles in the gel boundary layer (C_g) may not necessarily be constant, as assumed by this model (Shirazi *et al.*, 2010). This model is only applicable if a linear relationship exists between permeate flux and concentration of the protein in the bulk solution (Millipore Application Note, 2013).

3.3.3.1.2 RESISTANCE IN SERIES MODEL

The resistance in series model relates permeate flux during membrane separation process to TMP and total hydraulic resistance by using Darcy's Law. The total hydraulic resistance is the summation of the resistance of the new membrane, resistance of the cake or gel layer and the resistance due to adsorption of solute to the membrane pores (Corbatón-Báguena *et al.*, 2015).

The permeate flux, J, of a solvent limited by the resistance in series model can be calculated using the following equation:

$$J = \frac{\Delta P}{\mu\,(R_m + \grave{R_a}\,(1 - e^{-bt}) + R_g}$$

J	=	Permeate flux
ΔP	=	Transmembrane pressure
μ	=	Solution viscosity
R_m	=	New membrane resistance
$\grave{R_a}$	=	Concentration polarization resistance
R_g	=	Cake layer resistance
b	=	Fouling rate

Equation 3:2: *Estimate of permeate flux according to the resistance in series model.*

3.3.3.1.3 OSMOTIC PRESSURE MODEL

This model can only be applied to systems that generate an osmotic pressure differential across the membrane due to high concentration of rejected solute particles at the membrane interface (Shirazi *et al.*, 2010). Therefore this model is only applicable to systems such as RO filtration and is not applicable to UF or microfiltration since the use of porous membranes renders the osmotic pressure negligible (Jonsson and Tragardh, 1990). The osmotic pressure model is based on the fact that osmotic pressure greatly increases as solute particles accumulate at the membrane interface during CP. The osmotic pressure model suggests that it is this pressure that limits the permeate flux during UF of proteins. The osmotic pressure model can be defined according to the following equation (Shirazi *et al.*, 2010):

$$J = \Delta P - \frac{\Delta \Pi}{\mu R_T^{`}}$$

J	=	Permeate flux
ΔP	=	Transmembrane pressure
Δπ	=	Osmotic pressure difference
μ	=	Solution viscosity
R_T	=	Total resistance

Equation 3:3: *Estimate of permeate flux according to the osmotic pressure model.*

Several well-known CP models include the film theory model and the Spiegler-Kedem model. The film theory model is based on the mass transfer of solutes across the membrane where the driving force is the chemical potential of the solute; this model is applicable to RO and NF separations (Shirazi *et al.*, 2010). The Spiegler-Kedem model is similar to the film theory model but also uses a reflection co-efficient to estimate permeate flux during CP conditions.

3.3.3.2 MEMBRANE FOULING

Membrane fouling is an inherent characteristic of UF of proteins and can be described as any form of flux decline that is attributable to changes on the surface or on the pores of membranes, caused by the deposition of solutes that ultimately results in a loss of permeate flux (Marshall *et al.*, 1993; Vincent-Vela *et al.*, 2012; Tijing *et al.*, 2015). This definition establishes the distinction between MF and CP: CP is affected by hydrodynamic conditions (shear rate, flow velocity and solution viscosity) and does not impact the physical properties of the membrane (Choe *et al.*, 1986; Marshall *et al.*, 1993), while MF (Figure 3:7) is not only impacted by the hydrodynamic conditions of the process but also by the type and concentration of protein being ultrafiltered, the type and characteristics (pore size, surface roughness, surface charge and hydrophobicity) of the membrane being used, the solution chemistry (e.g. pH and ionic strength) and hydrodynamic conditions (Lim and Mohammad, 2010).

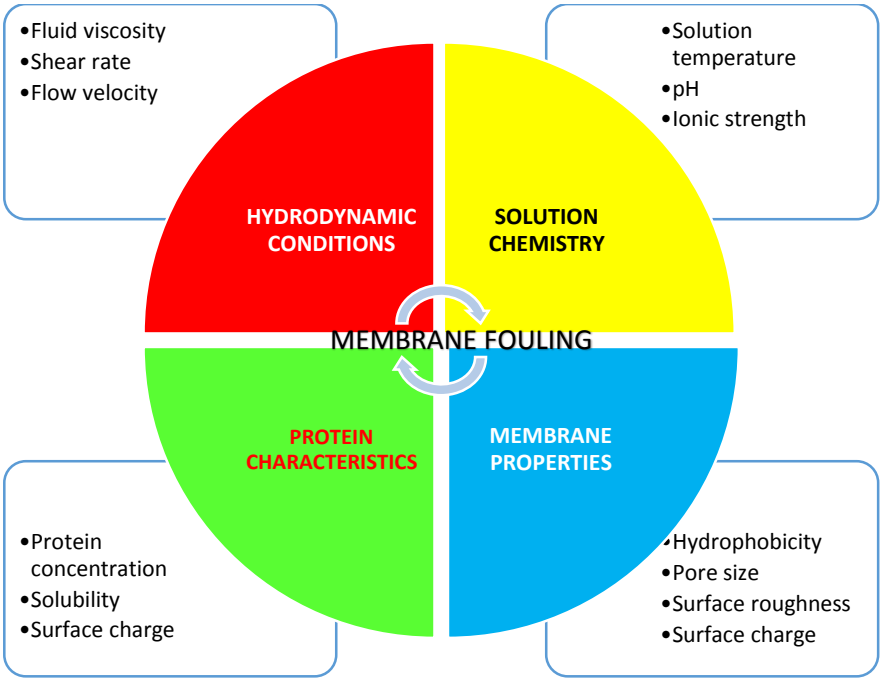


Figure 3:7: *Key factors, as described by Lim and Mohammad (2010), that influence membrane fouling including hydrodynamic conditions (shear rate, flow velocity); solution chemistry (pH, temperature); membrane properties (pore size, surface roughness and charge) and protein characteristics (concentration and solubility).*

There are two mechanisms by which MF generally occurs. In one mechanism, solutes adhere to the membrane by physic-chemical interactions, whilst in the second mechanism solute becomes entrapped in the membrane pores (Choe *et al.*, 1986). These changes to the surface of the membrane due to solute deposition were further classified into reversible effects, such as gel and cake layer formations, and irreversible effects such as adsorption (Vincent-Vela *et al.*, 2012). Protein adsorption to membrane surfaces is a result of a combination of physical interaction with membrane surface, electrostatic and hydrophobic interactions between solute components and the membrane and most importantly protein concentration (Schausberger *et al.*, 2009; Marshall *et al.*, 1993).

Marshall *et al.*, (1993) evaluated the impact of MF on the UF of proteins with respect to permeate flux, protein retention and selectivity. They also considered the impact of process parameters such as feed properties and operating conditions, on the UF of protein solutions. Following extensive investigation of the flux decline during UF of protein solutions, Marshall *et al.*, (1993) subdivided the process of flux decline during UF into three distinct phases namely (Marshall *et al.*, 1993):

- Phase one is characterized by a rapid drop in flux, primarily due to concentration polarization. Palecek and Zydney (1994) showed, by using electron micrographs, that the rapid drop in flux during this phase is also due to the adsorption of protein to the membrane surface thereby causing pore blocking or constriction
- In phase two, this drop in flux continues (although less rapidly than in phase 1), due to protein deposition on the membrane surface resulting in the formation of a protein monolayer. This protein monolayer is described as a dynamic layer, since the thickness and porosity of the layer depends on the type of protein being ultrafiltered and the related hydrodynamic conditions
- Phase three is characterized by a steady state equilibrium, where flux declines relatively slowly due to deposition of particles at the protein monolayer. In this phase it is assumed that the protein monolayer or gel layer is completed formed and remains consistent provide all hydrodynamic and electro-kinetic conditions remain the same. Palecek and Zydney (1994) documented a similar quasi-steady flux phenomenon

The earliest studies related to protein fouling or adsorption was conducted by Fane *et al.*, (1983), who described protein adsorption as a solute membrane interaction that results in a protein-membrane deposit, and which significantly impacts the rejection and flux characteristics of the membrane. MF and CP occur according to different mechanisms however, they are inter-related since both mechanisms are a function of protein concentration (Marshall *et al.*, 1993). Protein adsorption to membranes is a complex process and appears to be mainly irreversible.

3.4 INFLUENCE OF OPERATIONAL CONDITIONS ON ULTRAFILTRATION

3.4.1 pH AND IONIC STRENGTH

Several studies have confirmed the impact of pH and ionic strength on various membrane separation technologies including UF, by elucidating the electrostatic interactions between the components of UF, which are the solute (e.g. HSA), solvent and membranes. Ultrafiltration performance is dependent on the solution pH and ionic strength, since these factors influence the charge, stability and aggregation properties of the solute molecules, which in turn directly influence UF performance (Jonsson and

Tragardh, 1990). The pH and ionic strength of the feed solution plays a significant role in solute-solute interactions as well as solute-membrane interactions, by influencing the electrokinetic interactions between these components, with a direct effect on protein transmission and MF (Szymczyk and Rabiller-Baudry, 2012; Lim and Mohammad, 2010).

3.4.1.1 IMPACT OF pH AND IONIC STRENGTH ON PROTEINS

Swaminathan *et al.,* (1981) provided initial evidence that flux during UF is a function of pH. This group used various proteins in steady state and time dependent, stirred UF experiments, with various types of membranes, to prove the pH dependence of flux during UF. According to Swaminathan *et al.,* (1981), the findings of various authors who investigated the pH dependence of flux can be summarized as follows:

- A decrease in flux during UF of proteins, at all pH values, is evidently due to CP and MF that occurs during the process of UF
- In stirred UF experiments using BSA and other proteins, a decrease in flux as described in point above is noted. However, it is significant that the lowest flux has been observed for UF experiments conducted at the isoelectric point (IEP) of the protein of interest. Note that isoelectric point of a protein is defined as the pH at which that protein has no net surface charge i.e. surface charge equal to 0. The IEP of HSA is pH 4.7.
- The results obtained in unstirred UF of proteins is similar to that of stirred UF of similar protein solutions, using identical membranes and provide further evidence of the impact of pH on flux. Vilker, Smith and Colton (1975), using unstirred BSA UF, showed highest protein membrane surface concentration at the isoelectric point, when compared to other pH values
- Electron micrograph studies showed that the fouling deposits on membranes are dependent on pH.

Fane *et al.,* (1983) also conducted investigations into the UF of proteins at various pH and ionic concentrations. They used BSA as a model protein and aimed to determine permeate flux during UF across a range of pH and ionic concentrations. Further, the study aimed to measure the extent of protein fouling on the membrane. Fane *et al.,* (1983) noted a distinct minimum flux at the IEP (pH 5.0 – 5.5) of 1% BSA solutions,

these results were very similar to those noted by Swaminathan *et al.*, (1981). Further, they noted that in the presence of 0.2M NaCl, this minimum flux at the IEP was absent. However, in general, flux during UF of BSA was higher in the absence of salt (0.2M NaCl), except at the IEP, where the addition of 0.2M NaCl improved flux by more than 20%. They also noted that adsorption of protein to membranes increased with time and was greatest at the IEP. Also, adsorption of protein increases upon the addition of salt solution (0.2M NaCl) to the BSA. The results obtained were explained with respect to conformational change and charge effects of the protein, occurring at different pH and ionic environments, which impact the hydraulic permeability of the protein gel boundary layer and adsorbed protein. No quantitative data was presented in this study to support the claims of protein conformational changes or charge effects on solutes and membranes.

Palecek *et al.*, (1993) provided further evidence that supported the findings of Fane *et al.*, (1983). During the UF of BSA in ionic solutions, permeate flux decreases at pH above and below the IEP of the protein. The charge effects of proteins and the related impact on the permeability of the gel boundary layer was explained with reference to the relationship between ionic strength, Debye length and the force of repulsion between proteins. An increase in ionic strength results in reduction of the Debye length between proteins in solution; the reduction in the Debye length corresponds to a decrease in repulsion between proteins and thus proteins become tightly packed. The overall effect is the decrease in permeability of the gel boundary layer, resulting in loss of hydraulic permeability and therefore decrease in permeate flux. This paper elucidates the critical role of pH and ionic environment on UF of proteins. NBI carries out UF of HSA at pH7.0 – 7.2 and no salt is added during UF of HSA.

Scientists have proven, for numerous proteins e.g. BSA and whey protein, that the flux decreases at the IEP of the protein. Jonsson and Tragardh (1990) also suggested that at the IEP, the net charge on the protein is 0 and therefore the lack of electrostatic repulsion may result in the formation of tightly packed gel layers or increased adsorption onto the membrane. This decrease in flux at the isoelectric point may be in part, due to the decrease in solubility of proteins at the IEP (Swaminathan *et al.*, 1981).

Lim and Mohammad (2010) expanded on the effect of electrostatic forces during UF, as suggested by Jonsson and Tragardh (1990), by using gelatin (IEP approximately pH 5.3) as a model protein. Their study revealed the lowest permeate flux at pH values close to the IEP of the protein and increasing at pH values away from the IEP of the protein. The gelatin protein, at IEP has no net charge, and therefore repulsion between membrane and protein is at lowest – thereby favouring accumulation of protein at the membrane-protein interface. When the pH moves away from the IEP, electrostatic forces dominate the membrane-protein interactions resulting in decreased accumulation of protein at the membrane protein interface. In their study, Lim and Mohammad (2010) also showed the impact of NaCl of 0.1M concentration on permeate flux during the UF of gelatin protein solution. In this phase of the study a 4% (w/w) gelatin solution was subjected to UF at constant transmembrane pressure (TMP) of 2 bar, at varying pH. The results indicate that at pH above and below the IEP, a slight decrease in permeate flux was noted when compared to solutions at identical protein concentrations and pH equivalent to IEP. Interestingly, at IEP, the 0.1M NaCl salt solution improved permeate flux when compared to UF of similar solutions without 0.1M NaCl

The impact of pH, at fixed ionic concentration, on osmotic pressure was evaluated by Vilker *et al.*, (1980). An increase in osmotic pressure reduced the hydraulic flow through the semi-permeable resulting in low flux during UF of BSA. Vilker *et al.*, (1980) determined the osmotic pressure of increasing concentrations (84 – 475g/L) of BSA solutions at various pH (pH 4.5, 5.4, 7.4). Osmotic pressure was measured using a membrane osmometer cell and ionic concentration was maintained at 0.15M NaCl for all experiments. The results of the experiments indicate that osmotic pressure is dependent on solution pH and protein concentration. Generally, an increase in pH coupled with an increase in protein concentration results in increased osmotic pressures. The highest protein concentration evaluated (475g/L) measured an osmotic pressure 5 times higher at pH 7.4 than the osmotic pressure measure at the same protein concentration but at pH 4.5.

3.4.1.2 IMPACT OF pH AND IONIC STRENGTH ON MEMBRANES

McDonogh *et al.*, (1990) evaluated the effects of CP and MF of serum albumin at the membrane channel as a function of various operational parameters including pH. This group used radioactively labelled serum albumin to quantitatively evaluate MF during cross flow UF. The accumulation of protein in the membrane channel, caused by CP or MF, was determined as an increase in voltage caused by the radioactively labelled protein and measured by a scintillation detector. The results of investigation showed that the membrane surface accumulation of protein was strongest at the IEP of the protein and flux was lowest. The accumulation of protein at the membrane surface was greatly reduced at pH lower or higher than IEP.

In a study performed to evaluate the impact of pH on cleaning efficiency of thin film composite membranes with respect to ageing, Donose *et al.*, (2013) completed a very interesting baseline study on the effect of pH. Membranes were exposed to solutions at pH 4 and pH 1 for durations up to 20 hours. At pH 10 hydraulic permeability of the membrane increased by approximately 10% whilst at pH 4 hydraulic permeability of the membrane decreased by 4%. The rejection efficiency of the membrane remained unchanged.

3.4.1.3 INTERACTIONS BETWEEN PROTEINS AND IONS

The Hofmeister Series (HS) provided one of the earliest explanations for the interactions between macromolecules and ions. In 1888, Lewith and Hofmeister provided experimental evidence on the minimum concentration of salts required to precipitate proteins and thus the theories of solvation was born (Cacace *et al.*, 1997). Through the years several scientists have built on the founding theory of HS. The HS ranks ions according to their effects on physical behaviour of various molecules including proteins and colloids (Zhang and Cremer, 2006). The HS ranks ions into two categories namely kosmotropes and chaotropes. Kosmotropes exhibit strong hydration effects, stabilize proteins and cause salting out effects of proteins. Chaotropes exhibit strong hydrophobic effects and generally destabilize folded proteins and cause salting in effects.

The interactions between macromolecules (e.g. proteins) and ions or other molecules are defined by several theories. The Derjaguin, Landau, Vervey and Overbeek (DLVO) theory provided one of the earliest explanations for the interactions between molecules and there aggregation behaviour. The DLVO theory identifies molecules as point charges and explains the interactions of these molecules through determination of the interaction free energy which is characterized by the summative effect of attractive London-Van Der Waals's forces and repulsive electrostatic double layer interactions, between these molecules. The DLVO theory proposes that, as two identical molecules (similar surface charge and radius) approach each other, an energy barrier exists due to the repulsive force between the molecules and therefore prevents interaction between the molecules. If the interaction energy is high enough to overcome this energy barrier then the particles will collide and the London-Van Der Waal's forces will cause the molecules to adhere to one another resulting in aggregation of molecules. Several limitations of the DLVO theory is discussed in literature, the two most common are (Zhang and Cremer, 2006; Boström *et al.*, 2001):

- Inability to account for ion specificity (ions with similar charges have different effects in solution), such as that described by the HS, due to assumption that molecules act as point charges
- DLVO theory only works well for solutions with low salt concentrations and not at concentrations higher than 0.1M, which is the salt concentration for most biological systems. In solutions with salt concentrations lower than 0.1M, electrostatic interactions are dominant and the DLVO theory is pertinent to explaining the interactions between molecules and ions in this environment. However, in solutions with salt concentrations greater than 0.1M, electrostatic screening effects dominate the molecular interactions and the DLVO theory cannot be used to predict the subsequent interactions.

The stated limitations of the DLVO theory is generally explained through the interactions of several non-DLVO forces that act on molecules in solutions e.g. hydration force, hydrophobic force. Ninham and Yaminsky (1997) and Boström *et al.*, (2001) catered for specific ion effects in relation to the DLVO theory by understanding the impact of ionic dispersion forces in molecular interactions. These authors suggest that at high salt concentrations (>0.1M), the ionic dispersion forces are dominant and therefore the main predictor of aggregation behaviour of molecules.

However, they also indicate that all forces need to be considered to develop an analytical theory that predicts the behaviour of molecules in solutions or colloidal suspensions.

3.4.1.4 IMPACT OF pH ON ULTRAFILTRATION OF HSA

The impact of pH on protein stability and UF performance has been extensively studied. De La Casa *et al.*, (2008) showed that the pore radius of BSA at pH 7 to pH 9 was greatly increased, when compared to the pore radius of this protein at other pH. The large pore radius has a direct impact on protein rejection and protein transmission during UF of solutions containing proteins. The experimental evidence is clear that permeate flux is lowest at the IEP of the protein (Swaminathan *et al.*, 1981; Fane *et al.*, 1983; Palecek *et al.*, 1993; De La Casa *et al.*, 2008; Lim and Mohammad, 2010). The IEP of HSA is 4.7 and is stable at pH 7 (Fanali *et al.*, 2012).

The process step following UF of HSA is the final formulation of product prior to sterile filling into containers. Ideally, the product is required to be at neutral pH prior to formulation and sterile filling therefore, there is no benefit in evaluating optimization strategies based on variations in pH since it would require a further process step to adjust pH prior to formulation and sterile filling. Based on the information above this study will not evaluate the impact of pH on UF of HSA.

3.4.2 PROTEIN FEED CONCENTRATION

The feed concentration or concentration at which proteins are subjected to UF is critical to the efficiency of this unit operation in biotechnology processes. Protein concentration has a significant impact on MF by effecting both protein-protein interactions and protein-membrane interactions (Meireles *et al.*, 1991). High concentrations (varies for different types of proteins) of proteins in feed stocks (in conjunction with other factors, e.g. stirring speed, solution chemistry) are known to result in the denaturation of proteins, which is characterized by aggregation (irreversible and reversible) and polymerization (Roberts, 2014; Cromwell *et al.*, 2006; Kim *et al.*, 1993; Meireles *et al.*, 1991). The impact of denatured proteins, which form aggregates, on patients using plasma derived products include immunogenic reactions; similar to the antibody response exhibited by the human body when reacting to

microbial or bacterial pathogens (Rosenberg, 2006). This is especially important for manufacturers of PDMP's since immune responses to aggregated PDMP's will reduce the therapeutic effect of the drugs.

3.4.2.1 IMPACT OF PROTEIN FEED CONCENTRATION ON PROTEINS

Various authors have presented scientific evidence to suggest that denaturation of proteins plays an equally important role in MF and flux decline during UF, when compared to protein adsorption to membrane surfaces. In 1991, Meireles *et al.*, reported the impact of process conditions including feed concentration (and other process conditions including temperature, CFV, TMP) on albumin denaturation during UF. Meireles *et al.*, (1991), determined the concentration of protein that accumulates at the membrane solute interface (wall concentration) at a given TMP, using the osmotic pressure model. In using this model, they were able to theoretically determine wall concentration based on pressure profiles and the limiting permeate flux achieved during UF of proteins. The study revealed, for similar type membrane with different MWCO (10kDa, 40kDa and 100kDa), that a limiting flux always occurred at a wall concentration of 300g/kg (300g/L), for any set of operational parameters. Further, the wall concentration at which protein deposits were visibly noted on the membranes, was experimentally determined to be 400g/kg (400g/L). The film model suggested that the wall concentration is dependent on the concentration of the bulk solution; therefore an increase in bulk concentration ultimately results in increased concentration of solute at the membrane interface. The results obtained in this study are key points of interest for optimizing protein based UF processes in that it sets the boundaries for feed concentration at which UF can take place.

Most recently, Binabaji *et al.*, (2015) evaluated the UF of protein solutions at significantly high (225g/L) protein concentrations with the aim of determining the hydrodynamic and thermodynamic behaviour of proteins during this process. In this study a monoclonal antibody was used as the model protein and subjected to single step UF at constant feed flow rate (45mL/min) and constant TMP (1.03Bar) using a 30kDa regenerated cellulose membrane with membrane area equivalent to 0.88m^2. During the UF of monoclonal antibodies, filtrate flux was measured at various

concentrations of the protein. The data collected clearly indicated that permeate flux during UF of this protein decreases with an increase in protein concentration provided that key UF set points, e.g. feed flow rate, TMP are kept constant (Binabaji *et al.*, 2015).

3.4.2.2 IMPACT OF PROTEIN FEED CONCENTRATION ON MEMBRANES

In one of the earliest investigations into the impact of feed concentration on CP and MF, Reihanian *et al.*, (1983) evaluated the mechanism of fouling behaviour in UF of proteins using BSA as a test protein and several different types of membranes (polysulfone membranes of 30kDa; cellulosic membranes of 30kDa; polyion membranes of 10kDa). This study evaluated the impact of feed concentration on hydraulic permeability of membranes, in unstirred, dead end filtration systems (Reihanian *et al.*, 1983). The hydraulic permeability of the membrane, after exposure to increasing concentrations (0g/L to 10g/L) of BSA solutions was measured and analysed against initial hydraulic permeability, which was determined using pure water. This was done to determine the overall impact of feed concentration on hydraulic permeability of the membrane. The results showed that hydraulic permeability of the membrane decreased, with exposure to increasing concentrations of BSA solutions, thereby reducing permeate flux. These results also showed that the principle cause of MF is adsorption of protein molecules to the membrane surface.

3.4.3 ETHANOL CONCENTRATION

Organic solvents such as ETOH and acetone are commonly used as precipitating agents during the manufacture of pharmaceutical products (Shukla and Cheryan, 2002). Plasma fractionation methods that are based on the cold ETOH processes developed by Cohn *et al.*, (1946) use ETOH as a precipitating agent, to separate/fractionate various plasma proteins of interest, which are subsequently subjected to solid-liquid separation methods to extract the precipitated protein, e.g. centrifugation and/or membrane solvents are known to impact membrane performance during various membrane technologies including MF and UF.

3.4.3.1 IMPACT OF ETHANOL CONCENTRATION ON PROTEINS

In the primary fractionation of fresh frozen plasma, using the methods proposed by Kistler and Nitschmann (1962), ETOH is used in varying concentrations (from 19% v/v to 40% v/v) to precipitate protein fractions that will contain immunoglobulin (Fraction II paste) and albumin (Fraction V paste). In the purification of HSA (Figure 2:4), Fraction V paste is dissolved in WFI and ETOH (10% v/v). The dissolved albumin solution is depth filtered and then further refined through the process of UF. During UF, the dissolved albumin solution is dialysed using WFI at constant volume, to remove salts and lower the ethanol concentration to <1% (v/v).

Precipitation of plasma proteins with ETOH is conducted at low temperatures (between 0°C and 10°C), since organic solvents are known to denature proteins at room temperature (Jaffrin *et al.,* 1997; Cohn *et al.,* 1946). Traditionally, it was accepted that the mechanism of precipitation of plasma proteins using ETOH is due to a decrease in the dielectric constant of the solution, which increases the electrostatic forces of attraction between like protein molecules, thus precipitating these proteins out of the solution (Cohn *et al.,* 1946; Van Oss, 1989). C.J Van Oss (1989) proposed an alternate theory for the precipitation of plasma proteins by the admixture of WFI-ETOH. He suggested that at low temperatures, the dielectric constant of ETOH is close to that of water and therefore has a negligible impact on protein electrostatic interactions. Further, the major mechanism of ETOH precipitation of plasma proteins (at low temperatures) is due to the dehydration effect of the ETOH on the protein solution (since ETOH binds water more efficiently), which causes increased electrostatic attraction between protein molecules, resulting in precipitation of proteins. Pace *et al.,* (2004) evaluated the structural stability and solubility of proteins (using RNase SA as a model protein) in water and other solvents by determining the conformational stability of a test protein, as described by its free energy of the native and denatured states of that protein, in a particular solvent. They concluded that proteins would be extremely unstable and insoluble in polar solvents such as ETOH, to the extent that the degree of stability or solubility (or lack thereof) is dependent on the concentration of the solvent used.

The denaturing properties of ETOH on proteins were shown by Szymanska *et al.*, (2012), who evaluated the aggregation of lysozyme in ETOH (96%, v/v) admixtures according to the rheological properties (viscosity, shear flow) of ethanol-lysozyme solutions (all experiments were conducted at 25°C). This study showed that the viscosity of the ethanol-lysozyme solutions increased (approximately linear), with a corresponding increase in ETOH concentration up to 60% (v/v). For ETOH concentrations greater than 60% (v/v) the increase in viscosity of the solution was more significant and any minor increase in ETOH concentration prompted a large increase in viscosity of the solution. The increase in viscosity of lysozyme-ethanol solutions was caused by the aggregation of lysozyme protein due to conformational change of the protein induced by the increased ETOH concentrations. It is evident that increased concentration of ETOH in protein solutions may result in increased viscosity of the solution through denaturation of the protein. By the application of Darcy's Law, it is anticipated that such an increase in feed solution viscosity will result in overall decrease in permeate flux during UF of protein solutions (Szymanska *et al.*, 2012).

Jaffrin and Charrier (1994) investigated the optimization of refining processes for albumin production. Their research proved that increased concentration of ETOH in feed solutions reduced the permeate flux. In these experiments, albumin solutions of 30g/L containing no ETOH exhibited higher permeate flux than an albumin solution of 15g/L containing 40% (v/v) ETOH. In comparing the permeate flux of both solutions, the researchers noted an approximately 80% reduction in flux, measured across a range of CFV (monotonically increasing from 200 – 500L/h) and increasing TMP. All experiments were completed using a 10kDa polysulfone membrane and a 20kDa cellulose acetate membrane, both with membrane surface area of 0.5m^2. For both membrane types, the reduction in flux was explained by an increase in viscosity due to increased ETOH concentrations and the formation of a thicker gel layer. These findings reported in 1994 by Jaffrin and Charrier were confirmed by Gupta *et al.*, (1995), who investigated the impact of ETOH on UF of albumin solutions using mineral membranes. Gupta *et al.*, (1995) showed that the presence of ETOH in albumin feed solutions increased the viscosity of the solution and therefore reduced the permeate flux during UF of this protein. It is noted that an albumin solution of

50g/L containing 30% (v/v) ETOH exhibits lower permeate flux at similar TMP than an albumin solution of 50g/L containing 20% (v/v) ETOH (Gupta *et al.*, 1995).

Jaffrin *et al.*, (1997) showed that albumin solution of 20g/L containing no ETOH had increased permeate flux when compared to albumin solutions of similar concentration containing 20% ETOH. These studies reported in 1997 concurred with the earlier research performed by Jaffrin and Charrier (1994) and provided further proof that the permeate flux during UF of albumin with ETOH is independent of CFV and does not influence the kinetics of concentration polarization. The decline in flux due to high ETOH concentration was explained by the thickening of the gel boundary layer due to increase in the viscosity of the protein solution, based on the inverse relationship of membrane flux and solution viscosity, as defined by Darcy's Law. The viscosity (mPa.s) of albumin-ethanol solutions at 20°C and varying concentration of protein, is indicated in Table 3:3 to exemplify the impact of ETOH concentration on feed solution viscosity.

Table 3:3: *The viscosity (mPa.s) of solutions containing various concentrations of ethanol (0%, 20% and 30%). Note that viscosity increases as ethanol concentration increases, irrespective of protein concentration (Jaffrin et al., 1997).*

	PROTEIN CONCENTRATION (g/L)		
ETHANOL CONCENTRATION (v/v)	**0 (g/L)**	**10 (g/L)**	**20 (g/L)**
0 (%)	1.00	1.20	1.25
20 (%)	2.08	2.40	2.45
30 (%)	2.40	2.75	2.85

3.4.3.2 IMPACT OF ETHANOL CONCENTRATION ON MEMBRANES

In 1979, Nguyen *et al.*, provided scientific evidence for the impact of organic solvents on various types of UF membranes by evaluating the effect on hydraulic permeability of the membrane when exposed to organic solvents. In their study both water miscible solvents e.g. ethanol, methanol, butanol and acetone and water immiscible solvents e.g. benzene, toluene, heptane, decane and chloroform were used to measure hydraulic permeability on various types of membranes, in the absence of solutes. The hydraulic permeability of the membrane, derived from Darcy's Law, was measured as a function of the permeability ratio. This is a ratio of the permeability coefficient of the organic

solvent vs. the intrinsic permeability coefficient of the membrane using pure water, and is a measure of the effect of the organic solvent on the structure of the membrane (Nguyen *et al.*, 1979). A permeability ratio of greater than 1 suggests that the membrane has increased permeability in the presence of the organic solvent than compared to pure water. A permeability ratio equal to 1 suggests no change in membrane permeability when comparing organic solvent with pure water. The results of the research indicate that analytical grade (96%, v/v) water miscible solvents (ethanol, methanol, butanol and acetone) increased the permeability and therefore altered the structure of polysulfone membranes. Although no evidence was provided for the mechanism that resulted in increased permeability of the membrane, Nguyen *et al.*, (1979) suggested that the increase could be attributed to the solvation of the polymeric chains of the membrane network. Note that polysulfone membranes are generally the type of membrane used for UF of albumin at NBI. The permeability ratio for the polysulfone membrane was the highest measured for all types and equal to 3.6. This suggests that extremely high concentration (>90%, v/v) of ETOH in the feed solution, prior to UF of proteins, may impact the hydraulic permeability of the membrane and this may negatively affect membrane performance, since solute rejection may be lowered whilst flux during UF of proteins may increase. The ETOH concentration in the HSA solution prior to UF is approximately 10% (v/v) and might be too low to have any impact on membrane permeability. Further, the studies conducted by Nguyen *et al.*, (1979) were independent of solutes and therefore does not provide any evidence for combined impact of ETOH on membranes and solutes with respect to UF.

Lencki and Williams (1995) conducted similar studies to those of Nguyen *et al.*, (1979), and further attempted to quantitatively demonstrate the effect of solvents on flux behaviours of UF membranes. Lencki and Williams (1995) examined the effect of methanol, ethanol and acetonitrile solutions on the flow resistance (or hydraulic permeability) of cellulose and polysulfone membranes (10kDa and 30kDa MWCO). The experimental results were plotted in terms of relative resistance as a function of solvent concentration. The effect of solvent on membrane hydraulic permeability depended on the physical properties of the membrane as well as nature of solvent/membrane interactions. Also, the results showed that increasing

concentrations of ETOH (from 0% to 100%, v/v), increased the flow resistance of a 10kDa membrane.

3.4.4 TEMPERATURE

Temperature is a critical process parameter in UF of proteins due to its significant impact on membrane performance and protein fouling, since temperature is known to impact the physic-chemical properties of both the membrane and the protein of interest (Mo *et al.*, 2008). The solution chemistry and the thermodynamic state of a solution impacts the ability of proteins to maintain their stable, native conformations; any variation in these factors (solution chemistry and temperature) can lead to denaturation of proteins (Franzosa *et al.*, 2010; Farrugia and Pico, 1999). Temperature is not only a critical process parameter for UF of proteins but is also critical for primary plasma fractionation processes since the solubility characteristics of plasma proteins are greatly influenced by the temperature of the system they encompass (Cohn *et al.*, 1946).

3.4.4.1 IMPACT OF TEMPERATURE ON PROTEINS

Temperature is the most common physical factor that causes denaturation of proteins since slight variations in temperature impact the thermodynamic properties of the protein molecules causing them to transition from their native, three dimension configurations to several alternate transitional configurations (Grigoryan and Shilajyan, 2013; Park *et al.*, 2011). This alteration of the three dimensional structure of proteins at a certain temperature causes the denaturation of these molecules by the breaking of hydrogen bonds in the peptide resulting in a decrease in the solubility of the proteins. This decrease in the solubility of the proteins induces the aggregation of proteins (Park *et al.*, 2011).

The impact of temperature on proteins, especially albumin, has been well documented by several authors. During the early 1970's, Knut Wallevik (1972) explored the thermal denaturation of HSA under varying pH conditions and guanidine hydrochloride – a common protein denaturant. The polarimetric experiments employed by Wallevik (1972) showed that HSA, like most proteins, is highly stable at low temperatures, especially below 0°C. All folding and unfolding of the HSA molecules was reversible up to a temperature of 50°C and most significantly, α helices

content (which is a measure of conformational stability in the native structure) is lowest (26.8%) at high temperature (50°C) and highest (45.8%) at low temperature (2°C). This indicates the thermodynamic preference for native, stable confirmation of HSA at constant pH and solution chemistry. Wetzel *et al.*, (1980) not only showed the impact of temperature on α helical content of HSA but also correlated an increase in temperature with increase in β sheets which corresponds to denaturation of HSA. It was also noted that aggregation of HSA solutions by β sheet development at high temperatures where dependent on protein concentration, i.e. as protein concentration increased and temperature increased, the denaturing of the HSA protein occurred at lower temperatures. The results obtained by Wetzel *et al.*, (1980) were confirmed by Wu *et al*., (2000) who conducted heat-induced structural studies on HSA using infra-red spectroscopy and noted that during the thermal transition of HSA the protein undergoes distinct structural conversion from α helix (stable form) to β strands to β turns as temperature increases within a range of 45°C to 80°C.

Meireles *et al.*, (1991) determined the turbidity and flux of the retentate during UF of BSA at various temperatures (5°C, 8°C, 15°C, 22°C, 30°C) using set operating conditions of 100kPa, 1m/s feed circulation and 8g/kg protein feed concentration. In this study, turbidity was an indication of protein denaturation, as determined by size exclusion HPLC and light scattering experiments. At temperatures below 8°C, no increase in turbidity was noted whilst permeate flux decreased over a certain period of time and then remained in a steady-state condition. At temperatures greater than 8°C, an increase in turbidity of retentate is noted, as temperature increases. At 22°C, the flux does not achieve a steady state condition but rather continuously decreases during a time period of 140 minutes. These results suggest that an increase in temperature may result in denaturation of the protein resulting in MF that significantly inhibits permeate flux, during UF.

The denaturation and aggregation of proteins during UF at high temperatures (22°C, 30°C) noted by Meireles *et al.*, (1991) was explained further by Mo *et al.*, in 2008. This group evaluated the impact of various process conditions, including temperature on cross flow reverse osmosis system, using BSA as a test protein. They postulated that as the temperature (18°C, 25°C and 35°C) increases, the BSA molecule is unfolded to expose the hydrophobic centre core of the protein (contains the functional

groups of the protein) to the solvent. The solvent molecules attach to the functional groups of the protein thereby decreasing the electrostatic repulsion between BSA molecules and the interaction between the BSA molecule and the membrane. This results in aggregation and denaturation of the BSA protein, which corresponds to a decrease in permeate flux during UF of this protein. This denaturation of the BSA protein noted at higher temperature of 25°C and 35°C shows an increased decline in flux during UF of this protein at neutral pH i.e. pH = 7.0 (Mo *et al.,* 2008).

Darcy's Law states that the fluid flow through a porous membrane is dependent on the viscosity of the solution (Jonsson and Tragardh, 1990). Therefore, temperature is extremely influential during UF of proteins, since according to Darcy's Law we anticipate that as the temperature increases, a solution will become less viscous and will flow through the membrane with increased flux (Jonsson and Tragardh, 1990). The Arrhenius Equation correlates the relationship between viscosity and temperature and indicates that as the temperature increases, the viscosity of a solution decreases (Monkos, 1996). In a study conducted by Monkos (1996), he showed that the viscosity of solution containing BSA of concentration 335kg/m^3 (335g/L), decreased with increase in temperature. These experiments were completed in the temperature range 5°C to 45°C since it has been proven that HSA undergoes irreversible transformations at temperatures > 45°C. This irreversible transformation of HSA at temperatures greater than 45°C is also confirmed through studies conducted by Yadav *et al.,* (2014) and Rezaei-Tavirani *et al.,* (2006). This phenomenon is especially true during the microfiltration/clarification of fruit juices such as kiwi fruit, cherry juice and star fruit (Wang *et al.,* 2005). Similar studies to Monkos (1996) were conducted by Masuelli (2013). In this study, Masuelli (2013) studied the impact of temperature (35°C - 40°C), protein concentration (0.2%; 36.71%) on the viscosity (and other thermodynamic properties) of BSA. The intrinsic viscosity of BSA was analysed using the Huggins' method, which is defined as the ratio of viscosity to protein concentration, as protein concentration tends to zero. The results showed that as temperature increases, viscosity decreases for BSA solutions at both protein concentrations. The solution with higher protein concentration (36.71%) measured higher intrinsic viscosity of approximately 6.8cm^3/g when compared to the lower protein concentration (0.2%) which had an intrinsic viscosity of approximately 4.9 cm^3/g. In the microfiltration of West Indian cherry juice, Wang *et al.,* (2005) showed

the impact of temperature on permeate flux; at constant TMP and cross flow feed rates, the temperature of feed solutions was increased from 10°C to 40°C. This increase in temperature resulted in a decrease in viscosity from 148.9 cP.s at 10°C to 68.0 cP.s at 40°C which resulted in highest permeate flux (approximately 52.5LMH) at 40°C.

The strict application of Darcy's Law or the Arrhenius Equation may force one to operate UF of proteins at high temperatures, however, it is well known that high temperatures denature proteins through aggregation since the hydrophobic core of the protein becomes exposed to the solvent at high temperatures (Campbell *et al.,* 1993; Mo *et al.,* 2008). It is therefore preferable to complete UF of proteins at low temperatures to maintain protein stability and conformation.

The Darcy's Law does not consider the effect of CP and gel layer formation caused by the presence of solutes during UF. Thus for semipermeable or non-porous membranes the impact of temperature on flux can be derived from the osmotic pressure model (Jonsson and Tragardh, 1990). The osmotic pressure model suggests that the impact of temperature on flux is less pronounced than that described by the Darcy's equation, and is rather more dependent on concentration of the bulk feed solution. Further, it proposes that flux decreases as temperature increases since the increased CVF due to high temperatures inducing a less viscous solution, causes increased CP at the membrane surface due to the increase in convection of solute to the membrane, which in turn increases the osmotic pressure (Shirazi *et al.,* 2010; Jonsson and Tragardh, 1990). Further, high temperatures are known to cause denaturing and aggregation of proteins by initiating an unfolding of the proteins that exposes the inner, hydrophobic core of the protein to solvent molecules which increases aggregation and adsorption (Mo *et al.,* 2008).

3.4.4.2 IMPACT OF TEMPERATURE ON MEMBRANES

High temperatures are known to impact the polymeric structure of membranes and therefore negatively impact membrane performance (Kallioinen *et al.,* 2007). The impact of temperature on performance is normally detected by the non-linear increase in flux with increasing temperature. The PES membranes used in this study can

withstand temperatures up to 50°C and are considered mechanically durable to withstand extremes in temperature without compromising membrane performance.

3.4.5 PULSED ULTRAFILTRATION

Numerous techniques have been evaluated to reduce the impact of CP and MF during the process of UF, especially the UF of proteins. The techniques investigated include TMP pulsing (gas or solute), vibrating UF modules and cross flow flushing. Ma *et al.*, (2001) evaluated the effectiveness of various techniques (cross flow flushing, gas and water pulsation) in reducing MF. The results of their study confirmed the observations of other studies discussed below in that pulsation increased permeate flux by almost two fold and had a more significant impact on permeate flux when compared to other methods (Ma *et al.*, 2001). Transmembrane pressure pulsing is a technique that could be used to decrease CP and MF during UF of human serum proteins. The process optimization of refining of human serum proteins will involve an increase in protein concentration at the start of the refining process, it is therefore important to evaluate mechanisms, such as pulsing, to reduce the impact of MF and CP on permeate flux during UF.

In 1991, Rodgers and Sparks reported on a study to evaluate the impact of negative TMP pulsing on permeate flux, solute flux, solute retention and membrane selectivity. In this study, various types of membranes (polysulfone, polyvinylidene difluoride) were used and the impact of negative TMP pulsing on the parameters indicated above, was measured at different shear rates and pulse frequencies. The technique of negative TMP pulsing was first described by Rodgers in his DSc thesis of 1989; he concluded that a frequent, negative transmembrane force of significant amplitude can be used to reduce MF phenomenon such as adsorption and pore plugging whilst improving permeate flux rates and separation efficiency. This negative pressure primarily dislodges the gel layer that forms during ultrafiltration as well as any solute particles that adsorb or block the pores of the membrane. Further, this pulsing impacts the solute concentration at the membrane interface by aiding solute transport away from the membrane surface back into the bulk product. Most significantly, Rodgers and Sparks (1991) showed that pulsing at fixed shear rates of $700sec^{-1}$ and $160sec^{-1}$ and pulsing frequencies of 0.5Hz, 2Hz, 5Hz, increased total permeate flux by more than twice that of normal UF with no

pulsation. They attributed this increase in permeate flux to the reduction of CP and MF according to the mechanism described above; although they did not quantitatively determine the impact of pulsing on CP or MF. Further, they also noted that increased pulse frequency was required at increased feed bulk concentrations i.e. the impact of pulsing is reduced at high feed concentrations. During this study, pulsing also had a positive impact on solute flux at all shear rates. Rodgers and Sparks (1991) conclusively proved that negative TMP significantly increased permeate flux during UF, when compared to non-pulsed UF. However, no quantitative data were presented with respect to the reduction in CP or MF.

Rodgers and Sparks (1992) attempted to model the effect of TMP pulsing on CP. They attempted to formulate the parameters of this model, by determining if pulsing caused an alteration to CP and if so, which operating parameters significantly impacted the CP and the mechanism of CP reduction. In determining the impact of pulsing on CP, it was important to understand the dynamic development of CP during UF. The results of this study was in close agreement with that of the previous study in that pulsing during UF increased permeate flux, measured at similar conditions to previous work (0Hz, 0.5Hz, 2Hz and 5Hz; shear rate of 700sec-1 and 160sec-1). The key operational parameter that influenced effectiveness of pulsing was frequency of the pulse and a negative TMP during pulsing. Wilharm and Rodgers (1996) conducted studies to determine the significance of pulse duration and amplitude on cross flow UF of protein solutions containing BSA (1%) and immunoglobulin G (IgG – 0.3%). The results obtained during this study also showed that the frequency of the pulse, rather than pulse duration or amplitude, was the key operating parameter with respect to inducing increased permeate flux through TMP pulsing. Rodgers and Sparks (1992) further suggested that CP resistance is reduced by transmission of a force during pulsation that causes slight membrane motion which disrupts the CP gel layer. They referred to this model as the minimum flux model.

The previous studies noted in this literature review were directed towards determining the impact of pulsing on concentration polarization. Miller *et al.*, (1993) explored the impact of pulsing on MF. Previous studies (Rodgers and Sparks, 1992) indicated that pulsing had minimal impact on CP in dead end UF modules. Therefore Miller *et al.*, (1993) observed the impact of pulsing on solvent flux, in dead end batch cell system

using binary and single solutes. The results of their study showed that permeate flux measurements increased during pulsing, when compared to non-pulsed UF. Although the overall effect varied when compared to the cross flow UF, the overall effect of transmembrane pulsing i.e. increase permeate flux, was synonymous. They therefore concluded that transmembrane pulsing not only reduced the effects of CP but also MF.

3.5 CONCLUSION

The review of current literature available has revealed that indeed optimization of the UF of HSA is possible by analysing the impact of process and operating variables such as pH, temperature, back pulsing, ETOH concentration on UF of HSA. The overall goal of optimization strategy is to reduce CP and MF during UF of HSA, at the optimum operating parameters. Although a significant amount of literature is available on UF optimization strategies relating to proteins and related flux modelling concepts, the optimization of UF of HSA has not been significantly examined. However, it is important to note that much UF research has been completed by using BSA as a model protein which is very similar to HSA in terms of molecular structure and amino acid sequence.

During this study, ionic strength was varied within the range 0M to 3M. This broad range was selected to evaluate the effect of high ionic strength diafiltration diluents on permeate flux during UF of HSA, particularly at concentrations beyond the physiological range (0.1M). Literature informs us that ionic strength diluents of low molarity can decrease permeate flux during UF of proteins. However, in UF of HSA screening experiments, a minimal increase in flux was noted when ionic diluents were used during UF of HSA. Note that a key requirement for parenteral solutions is low Na content, therefore, if ionic solutions within the concentration range of 0M to 1M do indeed have an increasing effect on permeate flux during UF of HSA, then the study will have to evaluate a strategy to ensure that Na concentration is maintained at compliant specifications (<100mMol).

In the process optimization of the UF of HSA, the feed concentration of the protein is dependent on the dissolution volume of solvents required for this process. Equipment requirements e.g. feed tank size play a key role in UF process optimization strategies. It is therefore important to determine the maximum feed concentration at which to complete UF of proteins since this not only reduces the capital expenditure cost for equipment but also reduces the "UF equipment footprint" within the manufacturing facility. Most significantly, permeate flux reduction at high protein concentrations must be avoided due to the increased economic costs associated with increased processing times, cleaning and replacement of membranes – which is due to CP and MF. Thus the evaluation of the optimum protein feed concentration for the UF of HSA is of vital importance to this optimization strategy. A further benefit of the determination of optimal protein feed concentration is to ensure that the trade-off between diafiltration diluent and process time is adequately achieved; thereby influencing the productivity of the UF of HSA. During this study, protein feed concentration was varied within the range 60g/L to 220g/L. It was noted in UF of HSA screening experiments (data not shown), that membrane retention of HSA was <90% for protein feed concentrations >220g/L. These results established the upper limit for protein concentration. Literature review suggests that as protein feed concentration increases, permeate flux during UF of the protein decreases therefore, lower protein feed concentrations are favoured for UF of HSA. The lower limit for protein feed concentration is limited by the Fraction V paste dissolved in a fixed volume of WFI-ETOH admixture. An analysis of current process data indicates that the protein concentration for a defined mass of Fraction V paste in a fixed volume of WFI-ETOH is approximately 60g/L. Therefore, the lower limit for protein feed concentration was set at 60g/L.

Currently, Fraction V paste containing HSA, is dissolved in an admixture of purified water containing ETOH of final concentration 10% (v/v); not only is this a regulatory requirement but also has an impact on subsequent process steps namely depth filtration. A key point of this study is to evaluate the impact of ETOH concentration on UF of HSA performance and protein stability since the literature review has clearly established that ETOH has a significant impact on UF membranes, protein structure and UF performance. The performance of UF and stability of proteins in ETOH at various concentrations are closely linked to temperature. Therefore, the combined effect of temperature in

conjunction with ETOH concentration, on UF performance and protein stability, was also evaluated as part of this study. During this study, ETOH concentration was varied within the range 1% (v/v) to 10% (v/v). The required ETOH concentration during dissolution of Fraction V paste will never exceed 10% since this is the ETOH concentration at which glycoproteins, present in the dissolved Fraction V paste at low concentrations, will be precipitated out of solution. Further, this concentration of ETOH in the protein feed material ensures no freezing of this solution during depth filtration, which is conducted at a temperature of -3.5°C ± 0.5°C. Lastly, increased concentrations of ETOH (>20% v/v) are known to increase the permeability of PES membranes. Provided that scientific evidence confirms an improvement in UF performance, resulting from a change in ETOH concentration, the regulatory requirements stated above may be challenged.

Temperature is a key factor that influences the UF of proteins since CP and MF are modulated by temperature. Temperature of the protein solution impacts viscosity and the back diffusion of proteins away from the gel boundary layer. Further evidence is provided to show that ETOH in conjunction with temperature of the protein feed material has an impact on UF performance. The literature review confirms that stability of proteins is increased at low temperatures whilst higher temperatures will lead to denaturation (reversible and irreversible) of proteins. Although the temperature profile of HSA indicates irreversible denaturation of this protein at >45°C, the inclusion of inorganic solvents such as ETOH will further increase the instability of the protein at high temperatures (>25°C). Lastly, current UF of HSA process temperatures do not exceed 20°C ± 2.0°C. Therefore, considering the stability of the protein of interest and the solution matrix, the selected temperature range for UF of HSA used in this study was 5°C to 25°C.

Note that although pulsed UF was discussed in Section 3.4.5, it was not selected as a factor for this study since the UF rig that will be used for the UF of HSA will have to be modified to accommodate pulsed UF techniques. This will require capital expenditure to modify the equipment; following a careful cost benefit analysis it was confirmed that pulsed UF will not be included in this study, but will considered in all future work. The literature survey confirms the importance of the selected key factors on UF of HSA.

4 METHODOLOGY

4.1 DESIGN OF EXPERIMENTS

The key benefit of DOE is that it eliminates the OFAT approach and demonstrates any interactive effects between factors (varied according to the specific level for each factor) with reduced number of test runs. The CCD statistical methodology was used to design the experiments, evaluate the significance of each factor against the output variables and determine the optimum conditions for UF of HSA. Following a careful evaluation of the current NBI process, extensive literature review and several screening experiments, it was determined that the key factors that influence permeate flux during the UF of HSA at NBI are protein concentration (g/L); ionic strength (M); temperature (°C) and ethanol concentration (%). The levels for each factor was selected based on the literature review, regulatory guidelines, GMP compliance, screening experiments, equipment limitations and current, validated process parameters, the ranges of the level for each factor is indicated in Table 4:1.

Table 4:1: *Table indicating the various factors and their corresponding levels.*

INPUT VARIABLE/FACTOR	MINIMUM STAR POINT	MID LEVEL	MAXIMUM STAR POINT
Protein feed concentration (g/l)	60g/L	150g/L	220g/L
Ionic strength (M)	0M	1.5M	3M
Temperature (°C)	5°C	15°C	25°C
Ethanol Concentration (v/v)	1%	5.5%	10%

The minimum star point and maximum star point were defined for each factor and by using CCD, the experimental design matrix/array (Table 4:2) consisting of twenty six experiments including two repetitions at the central point (experiment number 25[c] and 26[c]) was generated. The order in which experiments where completed was randomized to reduce the effects of bias and to ensure statistical independence of the data collected.

Table 4:2: *Table showing experimental matrix/array including levels for each factor per experiment. The levels for each factor are varied within the ranges specified in Table 4:1.*

STANDARD RUN	PROTEIN CONCENTRATION (g/L)	IONIC STRENGTH (M)	TEMPERATURE (°C)	ETHANOL CONCENTRATION (%, v/v)
1	100.00	0.75	10.00	3.25
2	100.00	0.75	10.00	7.75
3	100.00	0.75	20.00	3.25
4	100.00	0.75	20.00	7.75
5	100.00	2.25	10.00	3.25
6	100.00	2.25	10.00	7.75
7	100.00	2.25	20.00	3.25
8	100.00	2.25	20.00	7.75
9	180.00	0.75	10.00	3.25
10	180.00	0.75	10.00	7.75
11	180.00	0.75	20.00	3.25
12	180.00	0.75	20.00	7.75
13	180.00	2.25	10.00	3.25
14	180.00	2.25	10.00	7.75
15	180.00	2.25	20.00	3.25
16	180.00	2.25	20.00	7.75
17	60.00	1.50	15.00	5.50
18	220.00	1.50	15.00	5.50
19	140.00	0.00	15.00	5.50
20	140.00	3.00	15.00	5.50
21	140.00	1.50	5.00	5.50
22	140.00	1.50	25.00	5.50
23	140.00	1.50	15.00	1.00
24	140.00	1.50	15.00	10.00
25 (C)	140.00	1.50	15.00	5.50
26 (C)	140.00	1.50	15.00	5.50

The key output/variable responses that were measured during this study can be broadly defined as membrane performance (Table 4:3). Membrane performance was determined by measuring permeate flux (LMH) during UF of HSA, membrane flux recovery after UF (%) of HSA and protein retention (%). Permeate flux was measured during all the steps of UF of HSA, membrane flux recovery was measured before and after each experiment and protein retention was analytically determined for diafiltration step five.

Table 4:3: *The output variables that were measured during these experiments were permeate flux (LMH), membrane flux recovery after UF (%) and protein retention (%).*

ULTRAFILTRATION PERFORMANCE	
OUTPUT VARIABLE	**UNITS**
Permeate flux	LMH
Membrane flux recovery after UF	%
Protein retention	%

The UF of HSA was classified into distinct steps (Figure 4:1) namely first concentration step, diafiltration steps one to five and second concentration step. The permeate mass flow rate data for each step was converted to volumetric flow rate, using specific gravity of 1, which was experimentally determined by weighing a fixed volume of permeate. Permeate weights varied from approximately 1.02g/mL when WFI was used as diafiltration diluent to 1.05g/mL when high concentration (3M) ionic strength diluents was used as diafiltration diluent. The permeate flux data for each experiment was statistically evaluated using the Fisher's Test for ANOVA. The desirability function for each factor was also statistically determined.

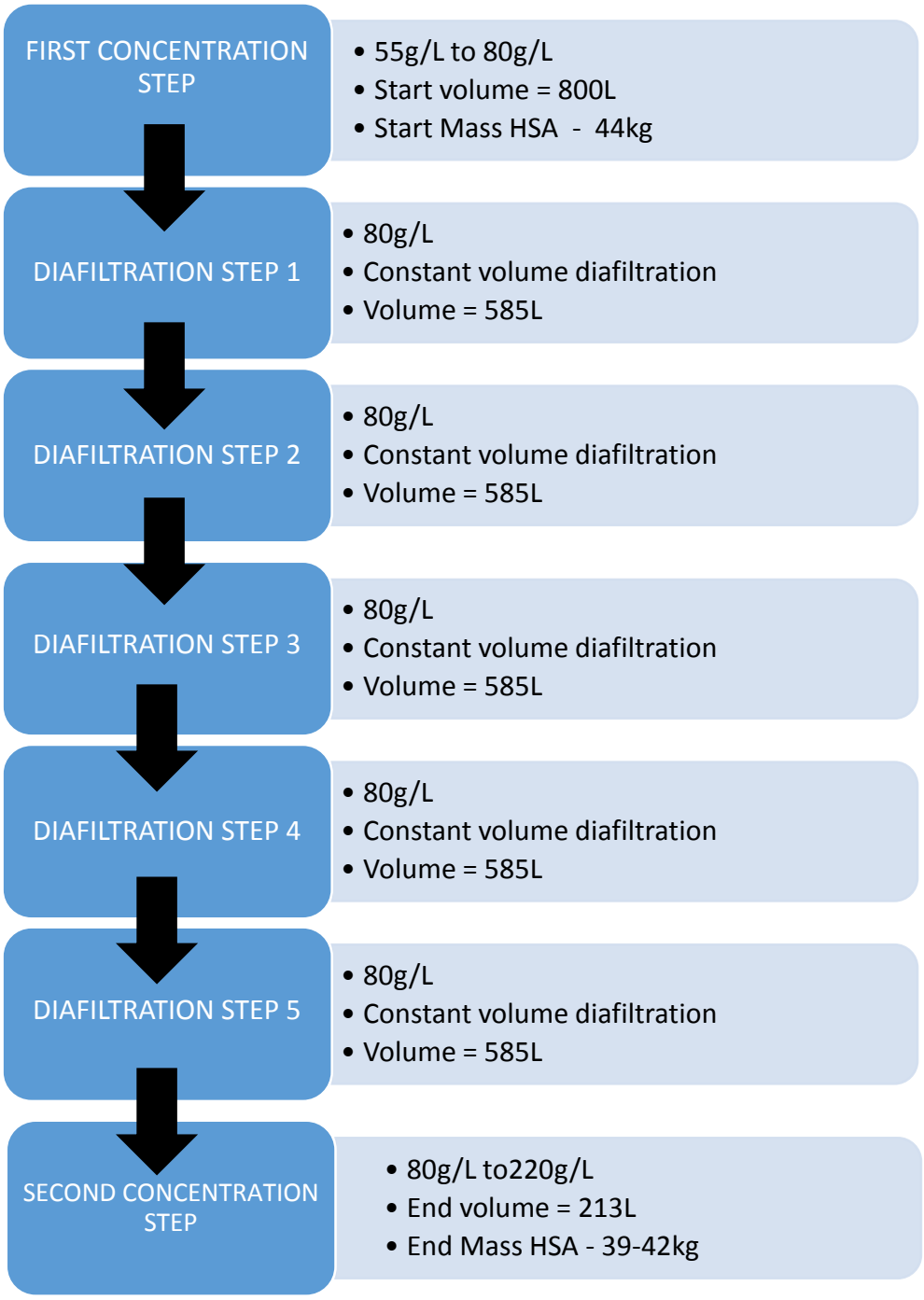


Figure 4:1: *Ultrafiltration is classified into distinct steps namely first concentration step, diafiltration steps one to five and second concentration step. The protein concentration (g/L) and volumes are indicated for each step of ultrafiltration as per the current NBI process. Also, typical start and end masses of HSA are shown to provide an indication of the product recovery (>85%) for the current manufacturing process.*

4.2 MATERIALS AND REAGENTS

4.2.1 MEMBRANE CASSETTE

The membrane cassette used in this study was the 10kDa, T series Centramate cassette supplied by Pall Corp LTD. This hydrophilic, anisotropic, PES membrane has low protein binding characteristics with high protein selectivity and enhanced flux rates (when compared to similar type membranes from Pall Corp LTD). The operating temperature range of this membrane is -5°C to 55°C; membrane is compatible with both acids and bases within the pH range pH 2 – 14 and the maximum permissible TMP is 4 bar at 55°C. The membrane surface area is equivalent to $0.1m^2$. Appendix A describes the specifications and performance data including the quality certificate for the T series Centramate cassette which was used in these studies.

4.2.2 PREPARATION OF FEED MATERIALS

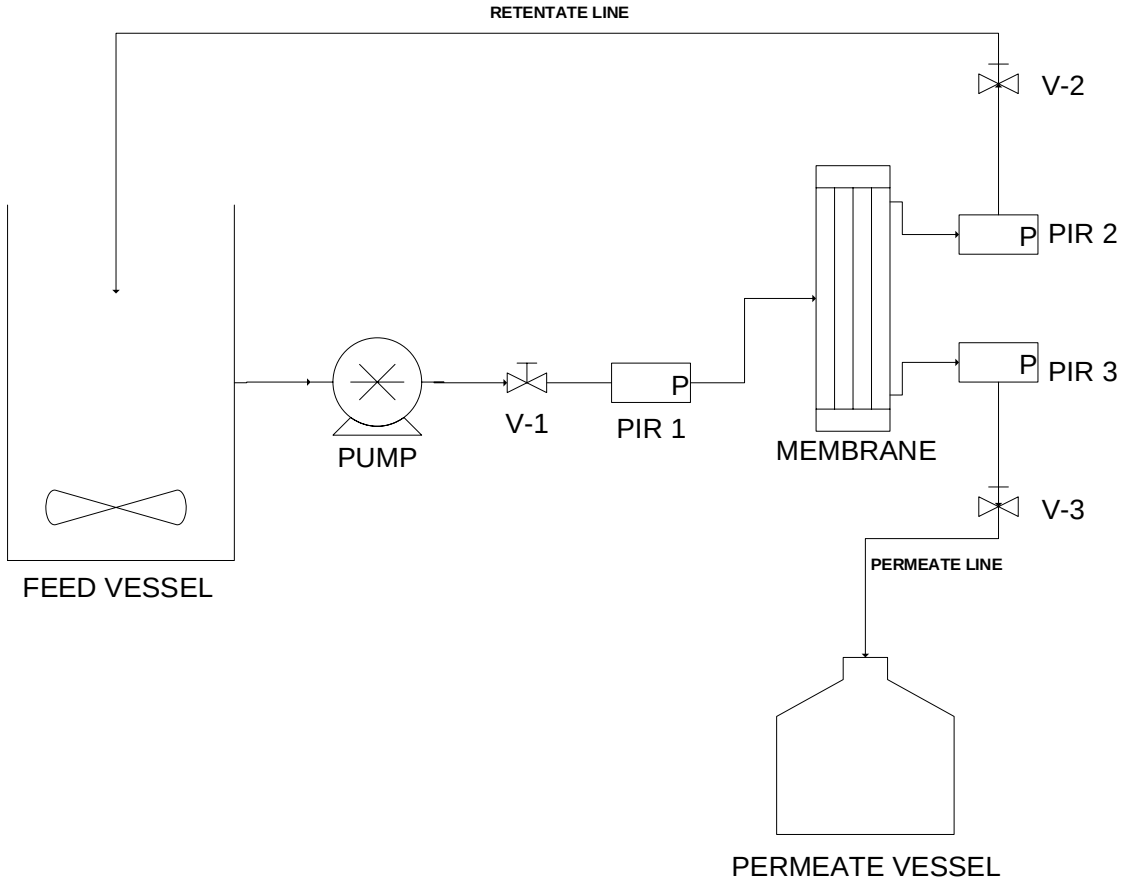
The Fraction V paste, containing HSA, used in the dissolution process was produced by NBI according to the methods described by Kistler and Nitschmann (1962); as referred in Chapter two, Figure 2:3. The dissolution of the Fraction V paste containing HSA, was based on the current NBI process described in Chapter two, Figure 2:4. Laboratory scale batches of HSA was prepared according to Appendix B; thereafter the feed material protein concentration and ETOH concentration was analytically determined by the A280nm method and gas chromatography method respectively. Aliquots of this feed material was used for each experiment; protein concentration was volumetrically adjusted during the first concentration step and ETOH concentration was adjusted using analytical grade ETOH (96% - v/v) supplied by Merck Millipore. Following the first concentration step, a sample of the feed material was retrieved and tested for protein concentration and ETOH concentration, as previously described.

4.2.3 PREPARATION OF REAGENTS

Sodium chloride (NaCl) solutions were prepared in WFI using analytical grade NaCl crystals supplied by Macco Organiques (Czech Republic). Sodium hydroxide (NaOH) cleaning solutions were prepared by dissolving NaOH pellets in WFI. The analytical grade NaOH pellets were supplied by Merck Millipore (Germany). All reagents were freshly prepared for each experiment.

4.3 EXPERIMENTAL SET UP

A typical, laboratory scale TFF unit with cassette holder and accessories was used to complete HSA experiments. Figure 4:2 is a schematic representation of the laboratory scale TFF unit used during this study. This unit was purchased from Pall Corp LTD and is designated the PE Centramate Holder. The holder is fabricated from stainless steel whilst associated connectors are fabricated from hard plastic. The holder and associated connectors were assembled according to instructions outlined by the supplier. The holder is equipped with three pressure gauges; one each for the permeate line, retentate line and feed line. All pressure gauges are manufactured locally (Guth SA) and calibrated annually. Transmembrane pressure of 1 ± 0.2 bar was maintained through all experiments by adjusting CFV and using a clamp on the retentate line. The experimental setup included a 2L plastic beaker as feed vessel. The feed solution in the beaker was continuously stirred using a bench stop mechanical stirrer with propeller type agitator (three blades connected to main shaft). A four piston diaphragm pump, Quattroflo 1200S, was used to generate the driving force for the UF of protein solutions. This positive displacement pump is designed to pump plasma proteins such as HSA and the pump chamber has no moving parts therefore heating of product or shedding of pump particles is negligible. Pump gaskets are FDA pharmaceutical classified, ethylene propylene diene monomer (EPDM) type. The Quattroflo 1200S has a maximum discharge pressure of 8 bar (room temperature) and a maximum CFV of 1200L/H at 0 bar. A Watson-Marlow, 4 head, peristaltic dosing pump was used to maintain constant volume during diafiltration, by addition of diafiltration diluent. The temperature requirements for each experiment was achieved to within ±2°C of the experimental specified temperature by using a Labotec heater chiller unit (Model No FTC300T). Temperature during the experiments was determined using a calibrated thermometer. All experiments were completed using a single, 10kDa, T series Centramate membrane cassette supplied by Pall Corp LTD with a surface area of $0.1m^2$. Therefore, total membrane surface area used for the experiments completed in this study was $0.1m^2$.



PIR 1 – Infeed line pressure gauge

PIR 2 – Retentate line pressure gauge

PIR 3 – Permeate line pressure gauge

V – Tube clamp/valve

Figure 4:2: *Diagrammatic representation of a typical ultrafiltration equipment module set-up. All the components illustrated in this diagram where used in the laboratory scale equipment set-up during this study.*

4.4 EXPERIMENTAL PROCEDURES

4.4.1 INSTALLATION AND CONDITIONING OF MEMBRANES

The installation and pre-use conditioning of the cassettes are clearly defined in the Pall Life Sciences User Guide for Membrane Cassette Care and Use Procedures, R00640 Rev B and the Membrane Cassette Care and Use Procedures for Centramate and Centrasette Cassettes – T-Series Omega Membrane, USD 2453 Rev A 1.00 (Pall Corp, 2015). Membrane cassettes were removed from packaging and inspected for any defects. The plastic gaskets provided with the cassettes were removed and rinsed

thoroughly with WFI. The membrane was inserted into the cassette holder and tightened to a torque setting of 80 inch-lb. Once installed, membrane cassettes were flushed (retentate and permeate lines to waste) with 5L of WFI and a constant permeate flux of 8L/min/m^2 was maintained during this flushing step. Upon completion of the flushing step, fresh WFI was recirculated through the membranes for a minimum of 30 minutes at a permeate flux similar to the flushing step and at a maximum of 1 bar TMP. The membrane cassettes were then sanitized using 0.5M NaOH. A bulk solution of 5L, 0.5M NaOH was made, of which 2L was flushed through the membrane cassette and the remaining 3L was recirculated through the membrane cassette for 30 minutes at conditions similar to the WFI flushing step. The normalized water permeability (NWP) for the new membrane cassette was determined as described in section below. Thereafter, the system was checked again for leaks and the standard integrity test completed. The cassette was stored in 0.2M NaOH buffer, until further use.

4.4.2 NORMALIZED WATER PERMEABILITY

The NWP test is a key membrane performance indicator and is used to determine membrane permeate flux recovery before and after each UF experiment (determination of membrane permeate flux recovery is explained in detail in section 4.4.3). Therefore, the NWP test determines the effectiveness of cleaning and sanitization procedures and is thus critical in providing information on the physical properties of the membranes. NWP was determined as the permeate flux of WFI, flushed through the membrane at varying TMP (0.17 Bar, 0.34 Bar, 0.51 Bar, 0.68 Bar) up to 0.68 Bar, were permeate flux is calculated according to Equation 4:2. The permeate flux of the WFI was normalized to 20°C (Equation 4:1) using the temperature correction factors developed by Pall Corp LTD for this membrane since WFI is not temperature controlled and the temperature of the water impacts permeate flux.

$$NWP_{20^{\circ}C}(LMH) = J_W \times TCF_{20^{\circ}C}$$

Jw – Clean water (WFI) permeate flux (LMH)

$TCF_{20°C}$ – Temperature correction factor at 20°C

Equation 4:1: *Calculation of normalized water permeability at 20°C, using temperature correction factors provided by Pall Corp LTD.*

The NWP value for a new membrane is greater than 145LMH at 1 Bar TMP and for a used membrane approximately 145LMH at 1 Bar TMP. During the first use of the membrane, the NWP is high and will reduce to a more consistent level as the membrane is used continuously.

4.4.3 ULTRAFILTRATION PROCEDURE

In the UF experiments, TMP was maintained consistently at 1 ± 0.2 bar, with a CFV of 0.5-1.5m/s (see Appendix D for calculation of CFV). The CFV and TMP is within the range allowed for UF of HSA, according to current GMP, regulatory, process requirements and supplier guidelines. The CFV is the minimum recommended by Pall Corp LTD for UF of protein solutions using the T-series, membrane cassette. The level for each factor was maintained as pre-determined for each experiment according to the design matrix/array indicated in Table 4:2. Prior to each experiment, the storage buffer was removed from the membrane cassette by flushing with 5L of WFI. Thereafter, the NWP of the membrane was determined as described in Section 4.4.2. HSA UF experiments were completed at fixed temperature and protein concentration levels. Permeate flux was determined throughout all the steps of UF by periodically (5 minute intervals) weighing the mass of permeate collected in 1 minute. This mass flow rate of permeate collected was then converted to permeate flux (LMH – litres/m^2/hour) as follows:

$$J\ (LMH) = \frac{Q}{A} \times SG$$

J – Permeate flux (LMH)

Q – Flow rate (L/h)

A – Membrane area (m^2)

SG – Specific gravity

Equation 4:2: *Calculation of permeate flux (LMH) derived from the mass flow rate of permeate (g/min) during the steps of ultrafiltration*

Following the completion of the UF of HSA experiment the membranes were cleaned as follows:

- Membrane was flushed with 5L of WFI
- 5L of 0.5M NaOH was prepared in a 5L beaker using WFI. 3L of this solution was flushed through the membrane. Thereafter the remaining volume of solution was recirculated for a minimum of 30 minutes at a permeate flux of 8L/min/m^2 and at a minimum of 1 bar TMP. Following the recirculation, the remaining volume (approximately 1.8L) of 0.5M NaOH was flushed through the membranes.
- Fresh WFI of 5L volume was flushed through the membrane to remove the NaOH. Thereafter, the pH of the WFI flushed through the membrane was checked; if pH was approximately pH 7.0, fresh WFI was recirculated through the membranes for a minimum of 30 minutes at a permeate flux of 8L/min/m^2 and at a maximum of 1 bar TMP.
- NWP was determined using fresh WFI as described in section 4.4.2.

Once the cleaning of the membranes was completed and the NWP (section 4.4.2) determined, the membrane flux recovery was determined according to Equation 4:3 below:

$$Membrane\ flux\ recovery\ (\%) = \frac{L_P}{L_{P,0}} \times 100\%$$

L_p – NWP after cleaning

$L_{p,0}$ – Initial NWP

Equation 4:3: *Determination of membrane flux recovery as a function of NWP before and after ultrafiltration experiments.*

The membrane flux recovery (%) of ≥80% was considered as a suitable flux recovery that confirms that the membrane is sufficiently clean and is suitable for the next UF experiment. The membrane was stored in 0.1M NaOH, until next use.

During each experiment, samples of permeate were taken after each step of UF. These permeate samples were tested for protein concentration by measuring the absorbance at

A_{280nm} using the Amersham Biosciences Ultrospec 2100 Pro spectrophotometer. The protein concentration in the sample was calculated using Equation 4:4 below:

$$Concentration\ (mg/mL) = \frac{A_{280nm} \times L}{\varepsilon_{280}}$$

A_{280nm} = Measured UV absorbance at 280nm

L = Length of the curvette (cm)

ε_{280} = Sedimentation co-efficient of HSA (5.8g/L)

Equation 4:4: *Determination of protein concentration of permeate and feed samples using absorbance at 280nm.*

These permeate samples were tested to determine the protein retention efficiency of the membrane and to ensure that no protein was lost to the permeate stream. After diafiltration step five, an aliquot of the feed and permeate was tested for protein concentration, to determine membrane retention efficiency and loss of protein to the permeate stream at the end of constant volume diafiltration. The calculated protein concentrations for feed and permeate was used to determine the protein retention efficiency (%) of the membrane according to the following equation:

$$Protein\ Retention\ (\%) = 1 - \frac{C_p}{C_f} \times 100\%$$

C_p = Concentration in permeate

C_f = Concentration in feed

Equation 4:5: *Determination of protein retention (%) by determining the ratio of the concentration of the macromolecule/protein (HSA) in permeate to that in the feed.*

A protein retention efficiency of greater than 90% suggests that the membrane is retaining the protein of interest sufficiently.

5 RESULTS AND DISCUSSION

5.1 PERMEATE FLUX DATA

The average mass flow rate (g/min), calculated at the end of the first concentration step, each diafiltration step and the second concentration step is shown in Table 5:1. The average mass flow rate during all steps of UF of HSA is displayed as average (g/min). Note that the mass flow rates for all experiments were converted to permeate flux data and attached to this study as Appendix C. Specific gravity of permeate was determined to be approximately equal to 1.

Table 5:1: *Average permeate mass flow rate (g/min) per step of ultrafiltration for all experiments conducted in this study. Table shows the experiment/run number, date experiment completed, mass flow rates (g/min) for first concentration step (FCS), diafiltration step one (DFS 1), diafiltration step two (DFS 2), diafiltration step three (DFS3), diafiltration step four (DFS4), diafiltraton step five (DFS 5), second concentration step (SCS). The average permeate mass flow rate during ultrafiltration is represented by AVG (g/min)*

DATE	EXPERIMENT NO.	FCS (g/min)	DFS 1 (g/min)	DFS 2 (g/min)	DFS 3 (g/min)	DFS 4 (g/min)	DFS 5 (g/min)	SCS (g/min)	AVG (g/min)
28-Mar-15	1	45.44	43.51	45.86	45.86	47.04	47.04	38.13	44.70
02-Mar-15	2	32.57	35.48	37.24	38.81	38.81	38.81	35.29	36.71
01-Apr-15	3	46.31	39.40	43.81	44.39	44.49	44.69	37.21	42.90
22-Mar-15	4	38.99	40.57	42.34	43.22	43.51	43.51	36.84	41.28
31-Mar-15	5	40.76	36.46	35.13	35.08	35.08	34.69	26.31	34.79
16-Mar-15	6	32.04	34.10	35.28	36.06	36.26	36.46	34.84	35.01
08-Mar-15	7	48.75	47.04	44.69	44.69	44.69	44.69	36.88	44.49
20-Mar-15	8	34.76	39.00	40.57	39.40	41.75	38.81	36.01	38.61
01-Apr-15	9	17.39	17.39	18.44	18.61	18.85	19.03	18.00	18.25
12-Mar-15	10	18.86	15.26	17.38	17.84	18.11	18.20	16.91	17.51
10-Mar-15	11	36.81	22.97	24.83	25.64	25.97	26.38	24.88	26.78
30-Mar-15	12	33.24	25.03	26.20	25.57	26.16	26.78	26.30	27.04
22-Mar-15	13	25.78	17.46	18.28	18.37	18.43	18.58	19.67	19.51
27-Mar-15	14	21.17	15.80	17.02	17.48	17.77	18.15	17.31	17.82
14-Mar-15	15	30.54	21.87	22.64	22.87	22.96	23.41	21.11	23.63
02-Apr-15	16	21.50	16.23	18.38	18.92	19.15	19.35	18.06	18.80
10-Mar-15	17	43.01	52.92	52.63	52.33	52.33	52.33	41.80	49.62
29-Mar-15	18	18.26	11.65	11.92	12.55	12.67	12.91	0.00	11.42
11-Mar-15	19	30.83	24.70	24.70	24.40	23.96	24.40	20.92	24.84
23-Mar-15	20	30.62	29.60	29.20	29.40	29.40	29.40	24.89	28.93
27-Mar-15	21	28.96	24.93	27.33	27.98	29.30	30.27	26.42	27.88
09-Mar-15	22	37.75	33.71	34.10	34.40	34.69	34.69	31.78	34.45
04-Mar-15	23	36.76	36.16	33.71	32.93	33.32	32.54	26.89	33.19
31-Mar-15	24	31.31	24.92	27.24	28.13	28.84	29.26	27.00	28.10
13-Mar-15	25 (C)	36.24	31.16	31.75	31.75	31.75	31.75	27.70	31.73
09-Mar-15	26 (C)	30.01	29.99	30.97	31.16	31.36	31.56	19.60	29.24

5.2 MEMBRANE FLUX RECOVERY DATA

Membrane flux recovery after each UF experiment (Equation 4:3) was derived from the NWP, which is a function of permeate flux (Equation 4:2) at a defined TMP. The permeate flux for the NWP was measured using WFI at four different TMP (0.17 Bar, 0.34 Bar, 0.51 Bar, 0.68 Bar). This permeate flux was normalized to 20°C (Equation 4:1) using the temperature correction factor table supplied by Pall Corp LTD since WFI is not temperature controlled. Table 5:2 indicates the NWP, normalized to 20°C at a TMP of 0.68 Bar. The results for the normalized, NWP calculated from permeate flux at 0.17 Bar, 0.34 Bar and 0.51 Bar is not shown. NWP for the virgin membrane was high (approximately 225LMH, 20°C, 0.68Bar) and became consistent after several UF of HSA pre-experiments (experiments that are not part of the experimental design). The initial NWP ($L_{p,0}$) was measured at 119.52 LMH.

Table 5:2: *Membrane permeate flux recovery (%) data calculated from the nominal water permeability data determined before each ultrafiltration experiment, at a TMP of 0.68Bar.*

DATE	EXPERIMENT NO	NWP (20°C)	MEMBRANE FLUX RECOVERY (%)	NWP (20°C)/Bar
02-Mar-15	2	119.52		175.76
04-Mar-15	23	114.84	96.08	168.88
08-Mar-15	7	135.24	113.15	198.88
09-Mar-15	26	109.06	91.25	160.38
09-Mar-15	22	120.00	100.40	176.47
10-Mar-15	17	117.30	98.14	172.50
10-Mar-15	11	113.27	94.77	166.57
11-Mar-15	19	105.60	88.35	155.29
12-Mar-15	10	122.54	102.53	180.21
13-Mar-15	25	107.01	89.53	157.37
14-Mar-15	15	102.06	85.39	150.09
16-Mar-15	6	114.84	96.08	168.88
20-Mar-15	8	107.10	89.61	157.50
22-Mar-15	13	134.16	112.25	197.29
22-Mar-15	4	127.68	106.83	187.76
23-Mar-15	20	105.84	88.55	155.65
27-Mar-15	21	111.93	93.65	164.60
27-Mar-15	14	94.51	79.07	138.99
28-Mar-15	1	129.36	108.23	190.24
29-Mar-15	18	105.44	88.22	155.06
30-Mar-15	12	83.64	69.98	123.00
31-Mar-15	24	77.26	64.64	113.62
31-Mar-15	5	123.00	102.91	180.88
01-Apr-15	9	132.48	110.84	194.82
01-Apr-15	3	122.76	102.71	180.53
02-Apr-15	16	127.09	106.33	186.90

The data in table 5:2 indicates that the membrane flux recovery (%) was ≥80% for all experiments except for experiment 12 and 24. The analysis of this data indicated a drop in membrane flux recovery from experiments 18,with experiments 12 and 24 showing membrane flux recovery lower than the recommended 140LMH at 1 Bar TMP. Following experiment 24, the membrane was cleaned twice according to procedures outlined in section 4.4.3. Therefore, before experiment 5, membrane flux was restored to original values after cleaning the membrane twice. Experiments 18, 12 and 24 were completed at protein concentrations of 220g/L, 180g/L and 140g/L respectively. This suggests that increased cleaning of membranes is required for UF of HSA at high protein concentrations. The membrane flux recovery data shows that membrane age did not

negatively impact the performance of the membrane, provided that the membrane cleaning strategy is effective, especially at high protein concentrations (>140g/L).

5.3 PROTEIN RETENTION ANALYSIS

The protein retention analysis was determined from permeate and feed samples taken after diafiltration step five since this step represents the end of CVD. Permeate and feed samples were subjected to absorbance at 280nm to determine protein concentration according to Equation 4:4. The protein retention (%) of the membrane was determined according to Equation 4:5. Note that permeate samples for all steps during the UF of HSA, for all experiments, were taken and tested for protein concentration (data not shown).

Table 5:3: *Protein rejection (%) of the membrane used during ultrafiltration experiments. The results show that HSA was retained by the membrane with an efficiency >90%.*

DATE	EXPERIMENT NO	DIAFILTRATION STEP 5			
		A280nm	CP (g/L)	CF (g/L)	PROTEIN RETENTION (%)
02-Mar-15	2	0.040	0.007	84.50	99.99
04-Mar-15	23	0.348	0.060	126.30	99.95
08-Mar-15	7	0.170	0.029	86.50	99.97
09-Mar-15	26	0.164	0.028	122.40	99.98
09-Mar-15	22	0.147	0.025	122.60	99.98
10-Mar-15	17	0.138	0.024	51.00	99.95
10-Mar-15	11	0.274	0.047	159.60	99.97
11-Mar-15	19	0.057	0.010	136.70	99.99
12-Mar-15	10	0.600	0.103	163.70	99.94
13-Mar-15	25	0.052	0.009	120.60	99.99
14-Mar-15	15	0.341	0.059	160.10	99.96
16-Mar-15	6	0.048	0.008	91.90	99.99
20-Mar-15	8	0.030	0.005	87.50	99.99
22-Mar-15	13	0.179	0.031	88.70	99.97
22-Mar-15	4	0.000	0.000	154.00	100.00
23-Mar-15	20	0.087	0.015	122.30	99.99
27-Mar-15	21	3.000	0.517	123.70	99.58
27-Mar-15	14	1.296	0.223	153.30	99.85
28-Mar-15	1	0.048	0.008	92.90	99.99
29-Mar-15	18	1.180	0.203	195.80	99.90
30-Mar-15	12	0.534	0.092	161.60	99.94
31-Mar-15	24	0.797	0.137	120.00	99.89
31-Mar-15	5	0.091	0.016	93.00	99.98
01-Apr-15	9	0.773	0.133	160.30	99.92
01-Apr-15	3	0.091	0.016	103.30	99.98
02-Apr-15	16	0.121	0.021	171.90	99.99

The results in Table 5:3 indicate that the HSA protein is retained by the membrane with an efficiency >90%, for all experiments completed during this study. We conclude that the membrane is able to retain the protein of interest whilst allowing unwanted salts and solvents to pass through the membrane. More significantly, these results show that the membrane used during this study is able to retain HSA across the range of HSA protein concentrations tested during this study.

5.4 STATISTICAL ANALYSIS OF THE FACTORS THAT EFFECT THE STEPS OF ULTRAFILTRATION

The UF of HSA is divided in to seven key steps namely: first concentration step, CVD (diafiltration steps one to five) and the second concentration step. Constant volume diafiltration step is further broken down into five, consecutive steps. The end of each of these diafiltration steps are determined as the volume of permeate collected equal to the volume of the feed solution at the start of diafiltration i.e. if the volume of the feed solution after the first concentration step is 650mL, then each diafiltration will end after collection of 650mL of permeate. Thus the effect of each factor on permeate flux was evaluated over the seven steps of the UF of HSA, where the measured outputs from each process step represents a unique set of dependent variables. However, it is important to note that although the impact of each factor on permeate flux during UF of HSA will be evaluated for each step, the steps are not mutually exclusive and are dependent on each other since UF by nature, is a dynamic process where process efficiency of each step is dependent on the efficiency of the preceding step. The magnitude of the effects of each factor, that had a significant impact on permeate flux during the steps of UF of HSA is also discussed in the sections below. Note that the magnitude of the effects of each factor has been considered for a GMP compliant UF rig with maximum membrane surface area of $30m^2$. Therefore, the magnitude of the effects of significant factors that influence permeate flux during UF of HSA is calculated using a membrane surface area of $30m^2$.

5.4.1 STATISTICAL ANALYSIS OF THE FIRST CONCENTRATION STEP

During the first concentration step, the dissolved Fraction V paste is concentrated from an initial protein concentration to a pre-determined starting protein concentration at which CVD will begin. The average mass flow rates (g/min) recorded during the first concentration step for all experiments are presented in Table 5:1.

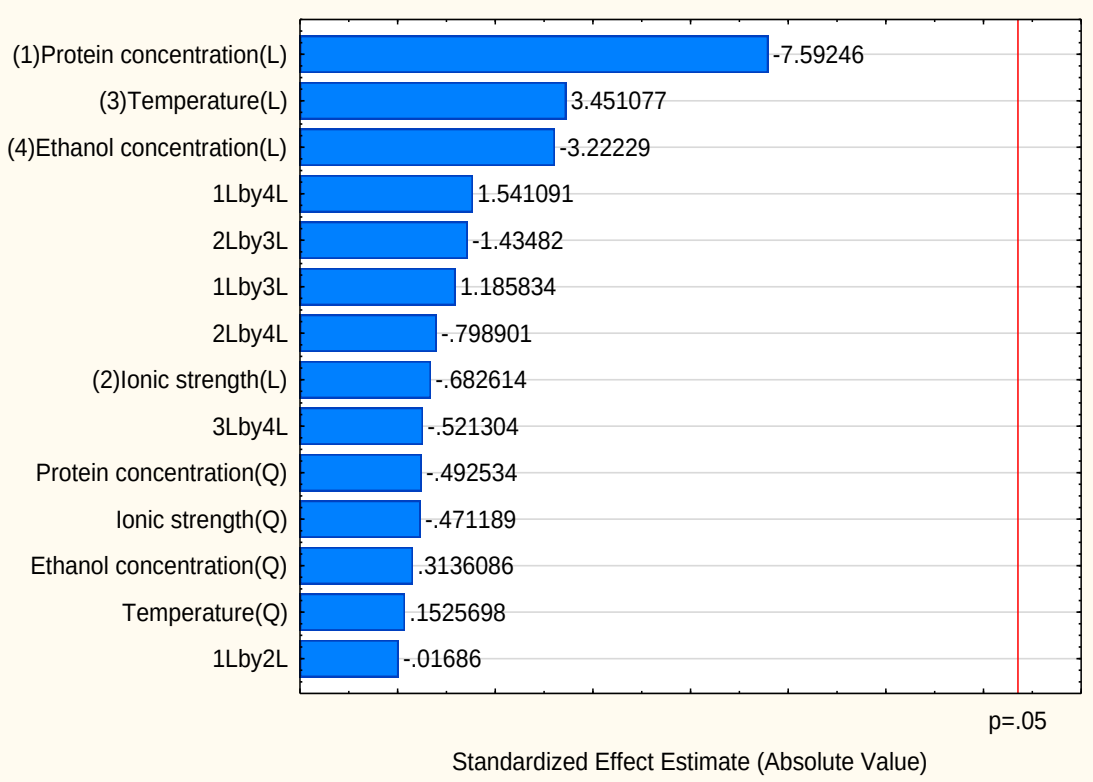


Figure 5:1: *Pareto chart of the standardized effects for the first concentration step of ultrafiltration. No factor had a statistically significant ($p < 0.05$) effect on permeate flux during this step of ultrafiltration.*

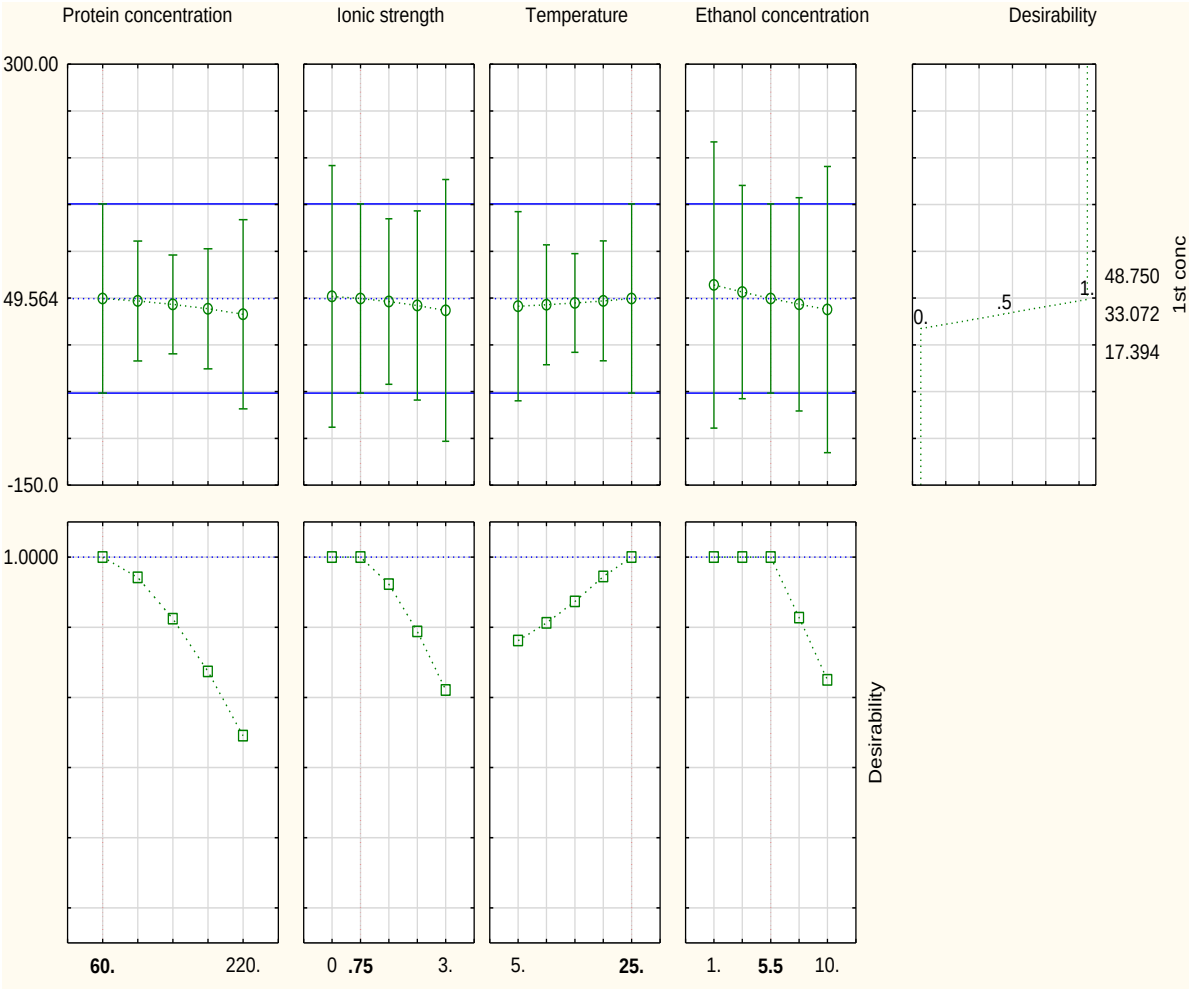


Figure 5:2: *Profile for predicted values and desirability for all factors for the first concentration step of ultrafiltration. The desirability function is maximized to achieve highest permeate flux for all factors, during the ultrafiltration of human serum albumin, were 1.000 is most desirable response and 0.000 is least desirable response. Since no factor had a significant impact on permeate flux during ultrafiltration, these values are not considered critical.*

Table 5:4: *ANOVA table for the first concentration step of ultrafiltration; indicating the p-value, Beta co-efficient, t value and 95% confidence intervals.*

FACTOR	EFFECT	STD. ERR. PURE ERR	t (1)	P	COEFF	STD. ERR. COEFF	-95% CNF LMT	+95% CNF LMT
Adjusted R^2 = 80.71%								
Mean/Interc	33.13	3.11	10.64	0.060	33.13	3.11	-6.44	72.69
(1) Protein concentration (L)	-13.65	1.80	-7.60	0.083	-6.83	0.90	-18.25	4.60
Protein concentration (Q)	-1.04	2.11	-0.50	0.709	-0.52	1.05	-13.91	12.87
(2) Ionic strength (L)	-1.23	1.80	-0.68	0.619	-0.61	0.90	-12.04	10.81
Ionic strength (Q)	-0.99	2.11	-0.47	0.720	-0.50	1.05	-13.89	12.89
(3) Temperature(L)	6.20	1.80	3.45	0.180	3.10	0.90	-8.32	14.52
Temperature(Q)	0.32	2.11	0.15	0.904	0.16	1.05	-13.23	13.55
(4) Ethanol concentration (L)	-5.79	1.80	-3.22	0.192	-2.90	0.90	-14.32	8.53
Ethanol concentration (Q)	0.66	2.10	0.31	0.807	0.33	1.05	-13.06	13.72
1L by 2L	-0.04	2.20	-0.02	0.989	-0.02	1.10	-14.01	13.97
1L by 3L	2.61	2.20	1.19	0.446	1.31	1.10	-12.68	15.29
1L by 4L	3.39	2.20	1.54	0.366	1.70	1.10	-12.29	15.69
2L by 3L	-3.16	2.20	-1.43	0.387	-1.58	1.10	-15.57	12.41
2L by 4L	-1.76	2.20	-0.80	0.571	-0.88	1.10	-14.87	13.11
3L by 4L	-1.15	2.20	-0.52	0.694	-0.57	1.10	-14.56	13.41

The analysis of variance (Table 5:4) of the permeate flux relating to the first concentration step for all experiments, revealed that none of the factors (temperature, protein concentration, ionic strength and ETOH concentration) had a significant impact on permeate flux during this step of the UF of HSA, within the specified level for each factor. Figure 5:1 is a graphical representation (Pareto chart) of the standardized effects of the factors on permeate flux during the first concentration step. This Pareto chart shows that all factors have a p-value greater than 0.05 ($p > 0.05$) and therefore all factors have no significant impact on the permeate flux during the first concentration step. Further, the t-value (Table 5:4) determined for all factors is approximately 0; this indicates little or no variation between the sample means of all the factors with respect to permeate flux. Note also that the interactive effects between factors did not have a significant impact on permeate flux during this step. The validity of the above conclusions are supported by the linearity of the normal probability plot vs. raw residuals (data not shown). The profile for predicted values and desirability (Figure 5:2) show distinct trends; however, these trends are considered not significant when interpreted in relation to the p-value.

The permeate flux during the first concentration step showed a rapid decline, for all experiments (data not shown). This decline in flux is in line with the fouling theory suggested by Marshall *et al.*, (1993) who suggested that the initial rapid decline in permeate flux at the start of UF of proteins is due to CP. Several other studies have also shown that reversible (gel and cake layer formation) and irreversible (protein adsorption) pore blocking mechanisms have contributed to this rapid flux decline at the start of UF of proteins (Verma and Sarkar, 2015; Vincent-Vela *et al.*, 2012). The continued decline in flux, during the first concentration step is also attributed to the increased adsorption of protein on the membrane surface resulting in the formation of a protein monolayer, referred to as cake formation.

The statistical analysis of the first concentration step revealed that none of the factors had a significant impact on permeate flux during this step of the UF of HSA, within the range of the factor selected for this study. This step (first concentration step) represents a volumetric reduction of the feed solution to achieve an acceptable protein concentration at which to complete CVD. Therefore, no diluent is added to the feed solution during this step. The CP and MF mechanisms responsible for decline in flux during this step are primarily driven by the hydrodynamic conditions, protein-membrane interactions and protein-protein interactions.

5.4.2 STATISTICAL ANALYSIS OF DIAFILTRATION STEP ONE

Diafiltration step one is the second step of the UF of HSA and the first step of CVD, using either WFI or NaCl as a diluent. The end of diafiltration step one was defined as the collection of permeate volume equal to one bed volume of the bulk solution (dissolved Fraction V paste) which was determined at the end of the first concentration step. The average mass flow rates (g/min) recorded during diafiltration step one for all experiments are presented in Table 5:1.

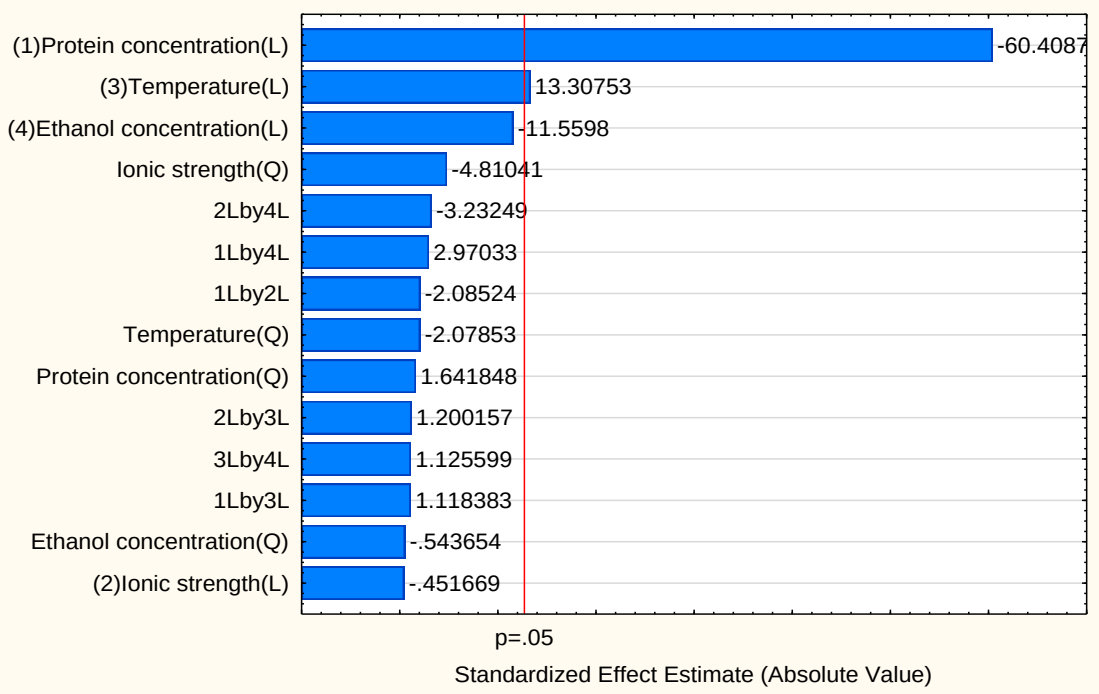


Figure 5:3: *Pareto chart of the standardized effects for diafiltration step one of ultrafiltration. Protein concentration and temperature had a statistically significant ($p < 0.05$) effect on permeate flux during this step of ultrafiltration.*

Table 5:5: *ANOVA table for diafiltration step one of ultrafiltration; indicating the p-value, Beta co-efficient, t value and 95% confidence intervals.*

FACTOR	EFFECT	STD. ERR. PURE ERR	t (1)	P	COEFF	STD. ERR. COEFF	-95% CNF LMT	+95% CNF LMT
Adjusted R^2 = 91.49%								
Mean/Interc	30.58	0.59	52.00	0.012	30.58	0.59	23.10	38.05
(1) Protein concentration (L)	-20.51	0.34	-60.41	0.011	-10.25	0.17	-12.41	-8.10
Protein concentration (Q)	0.65	0.40	1.64	0.348	0.33	0.20	-2.20	2.86
(2) Ionic strength (L)	-0.15	0.34	-0.45	0.730	-0.08	0.17	-2.23	2.08
Ionic strength (Q)	-1.91	0.40	-4.81	0.130	-0.96	0.20	-3.49	1.57
(3) Temperature(L)	4.52	0.34	13.31	0.048	2.26	0.17	0.10	4.42
Temperature(Q)	-0.83	0.40	-2.08	0.285	-0.41	0.20	-2.94	2.12
(4) Ethanol concentration (L)	-3.92	0.34	-11.56	0.055	-1.96	0.17	-4.12	0.19
Ethanol concentration (Q)	-0.22	0.40	-0.54	0.683	-0.11	0.20	-2.64	2.42
1L by 2L	-0.87	0.42	-2.09	0.285	-0.43	0.21	-3.08	2.21
1L by 3L	0.47	0.42	1.12	0.465	0.23	0.21	-2.41	2.87
1L by 4L	1.24	0.42	2.97	0.207	0.62	0.21	-2.02	3.26
2L by 3L	0.50	0.42	1.20	0.442	0.25	0.21	-2.39	2.89
2L by 4L	-1.34	0.42	-3.23	0.191	-0.67	0.21	-3.31	1.97
3L by 4L	0.47	0.42	1.13	0.462	0.23	0.21	-2.41	2.88

The analysis of variance (Table 5:5) of the mass flow rate relating to the diafiltration step for all experiments, revealed that protein concentration (t = -60.41, p= 0.0105, 95% CI[-12.41, -8.10]) and temperature (t = 13.31, p = 0.0477, 95% CI[0.10, 4.42]) had statistically significant impacts on permeate flux during this step of the UF of HSA. Ionic strength (t = -0.45, p = 0.7299, 95% CI[-2.23, 2.08]) does not have a statistically significant impact on permeate flux. ETOH concentration (t = -11.56, p = 0.0549, 95% CI[-4.12, 0.20]) does not have a statistically significant impact on permeate flux. However the substantive effect (Seltman, 2012) of ETOH concentration on permeate flux during this step of UF of HSA can be considered significant since the p-value (p = 0.0549) is close to p-value ≤ 0.05 and this factor has a corresponding reducing effect on permeate flux of 4.11g/min to 0.20g/min within a certainty of 95%. An increase in the protein concentration of 1g/L resulted in a decrease in the permeate flux between 12.41g/min and 8.10g/min. An increase in the temperature of 1°C resulted in an increase in the permeate flux between 0.10g/min and 4.42g/min. The adjusted R^2 value (Table 5:5) suggested that at least 91.49% of the variability in permeate flux during diafiltration step one was accounted for by taking into consideration the effects of protein concentration and temperature. The validity of the above conclusions are supported by the linearity of the normal probability plot vs. raw residuals (data not shown). Note that the interactive effects between factors (Figure 5:3) did not have a significant impact on permeate flux during this step.

Table 5:6: *Table showing the magnitude of the effects of the significant factors that influence permeate flux during diafiltration step one.*

Permeate Flux	**Protein Concentration (g/L)**	**Temperature (°C)**	**Ethanol Concentration (%)**
Co-efficient (β = +/-)	-10.25	2.26	-1.96
-95% (g/min)	-12.41	0.10	-4.12
+95% (g/min)	-8.10	4.42	0.19
-95% (ml/min)	-12.16	0.10	-4.04
+95% (ml/min)	-7.94	4.33	0.19
-95% (LMH)	-7.30	0.06	-2.42
+95% (LMH)	-4.76	2.60	0.11
L/\h (30m^2)	-218.92	1.80	-72.66
	-142.83	77.89	3.43

The magnitude of the effects of the significant factors that influence permeate flux during diafiltration step one are displayed in table 5:6. Protein concentration and temperature had a statistically significant impact on permeate flux during this step of UF of HSA. Ethanol concentration had a significant substantive effect on permeate flux. Protein concentration had an effect magnitude of -7.30LMH within the 95% confidence interval. This translates to a reduction of permeate flow rate of almost 220L/H, if 30m^2 of membrane area is used for the UF of HSA. Temperature has an effect magnitude of +2.60LMH within the 95% confidence interval, although the effect of temperature on permeate flux is smaller when compared to protein concentration. Ethanol concentration had a substantively significant impact on permeate flux during diafiltration step one; with an effect magnitude of -2.42LMH which is similar to the effect of temperature on permeate flux during this diafiltration step.

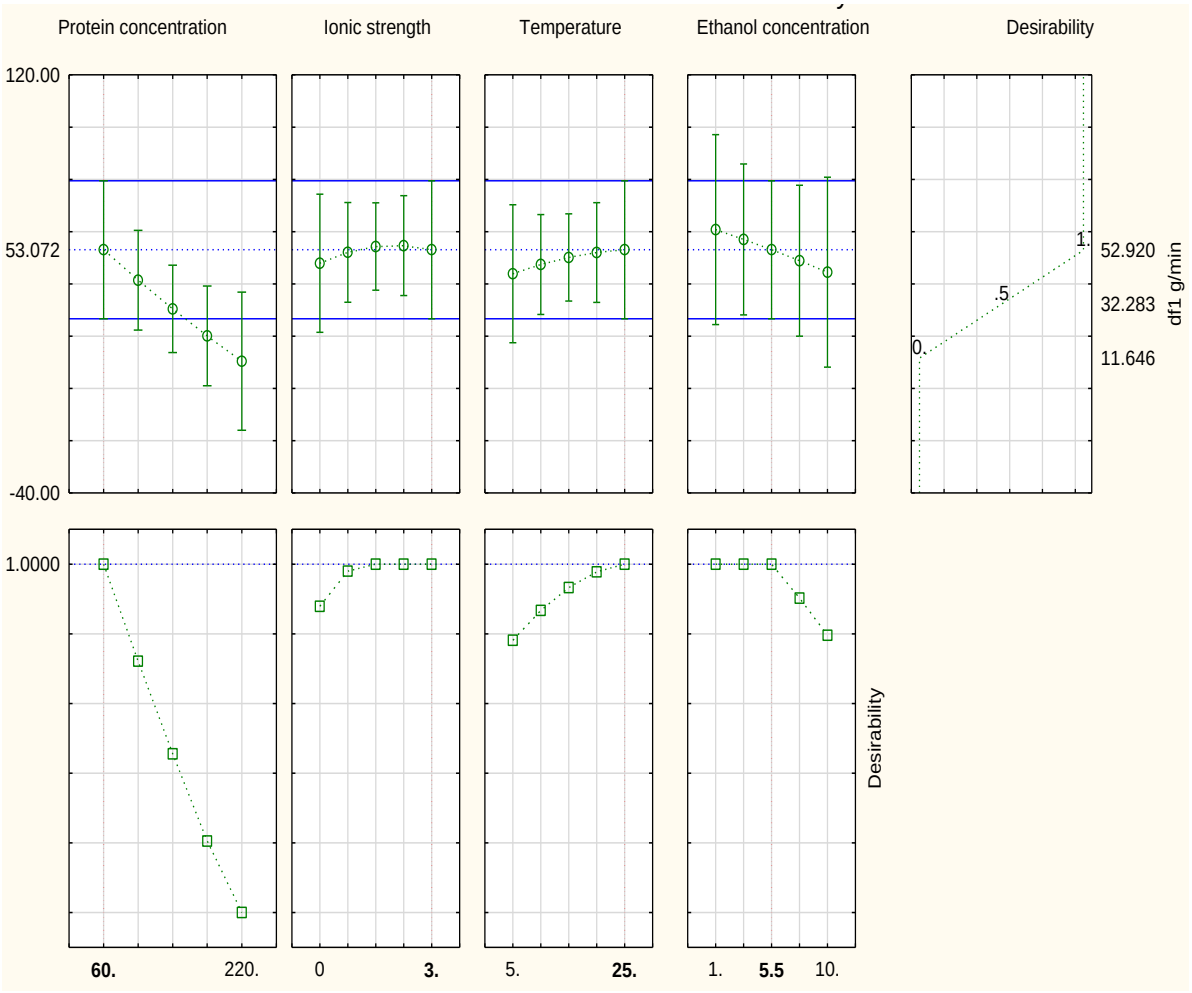


Figure 5:4: *Profile for predicted values and desirability for all factors for diafiltration step one of ultrafiltration. The desirability function is maximized to achieve highest permeate flux for all factors, during the ultrafiltration of human serum albumin, were 1.000 is most desirable response and 0.000 is least desirable response. The predicted value for protein concentration that achieves the higher permeate flux during diafiltration step one is 60g/L. The predicted value for temperature that achieves the highest permeate flux during diafiltration step one is 25°C.*

The permeate flux during diafiltration step one showed a consistent rate, for all experiments. This consistent permeate flux is in line with the fouling theory suggested by Marshall *et al.*, (1993), which proposed that a quasi-steady state flux is achieved following the formation of the gel boundary layer on the membrane surface. The statistical analysis of diafiltration step one revealed that protein concentration and temperature had significant impacts (Figure 5:3) on permeate flux during this step of UF of HSA. The profile for predicted values and desirability (Figure 5:4) showed that permeate flux clearly decreased as protein concentration increased. Further, the desired value for protein concentration during diafiltration step one is equal to 60g/L.

Membrane fouling and CP are dependent on protein concentration at the start of UF of HSA, therefore maximum permeate flux during this process is always achieved at the lowest protein concentration. The profile for predicted values and desirability also indicated that the permeate flux increased with an increase in temperature during this step of UF of HSA, with the optimum temperature for maximum permeate flux specified at 25°C, for diafiltration step one. It is also noted that a temperature of 20°C produced a desirability value close to +1.000 and may be considered when determining the optimum temperature set point for the optimization strategy for UF of HSA, considering the impact of temperature on protein stability. Ethanol concentration had a substantive significance on permeate flux during diafiltration step one, it was noted that increased ETOH concentration in the feed solution resulted in lower permeate flux during UF of HSA. The profile for predicted values and desirability declared a desired value within the range 0% (v/v) to 5.5% (v/v) for ETOH concentration, during this step of the UF of HSA. The primary goal of UF of proteins is to remove unwanted micromolecules (e.g. sodium, potassium and aluminium) and solvents (e.g. ETOH). During diafiltration step one the ETOH concentration is at a maximum when compared to other steps of UF of HSA, therefore, a significant turning point (5.5%, v/v) towards a decrease in flux (and therefore towards a non-desirable value of 0.000) is noted in the statistical analysis of the desired values for this factor. The profile for predicted value and desirability for ionic strength was not considered further, since this factor did not have a statistically significant impact on flux ($p > 0.05$).

5.4.3 STATISTICAL ANALYSIS OF DIAFILTRATION STEP TWO

Diafiltration step two is the third step of the UF of HSA and the second step of CVD; immediately following diafiltration step one. The end of diafiltration step two was defined as the collection of permeate volume equal to two times the bed volume of the bulk solution (dissolved Fraction V paste) which was determined at the end of the first concentration step. The average mass flow rates (g/min) recorded during diafiltration step two for all experiments are presented in Table 5:1. The statistical trends (Figure 5:5) between dependent and independent variables in the second diafiltration step are similar to those observed for diafiltration step one.

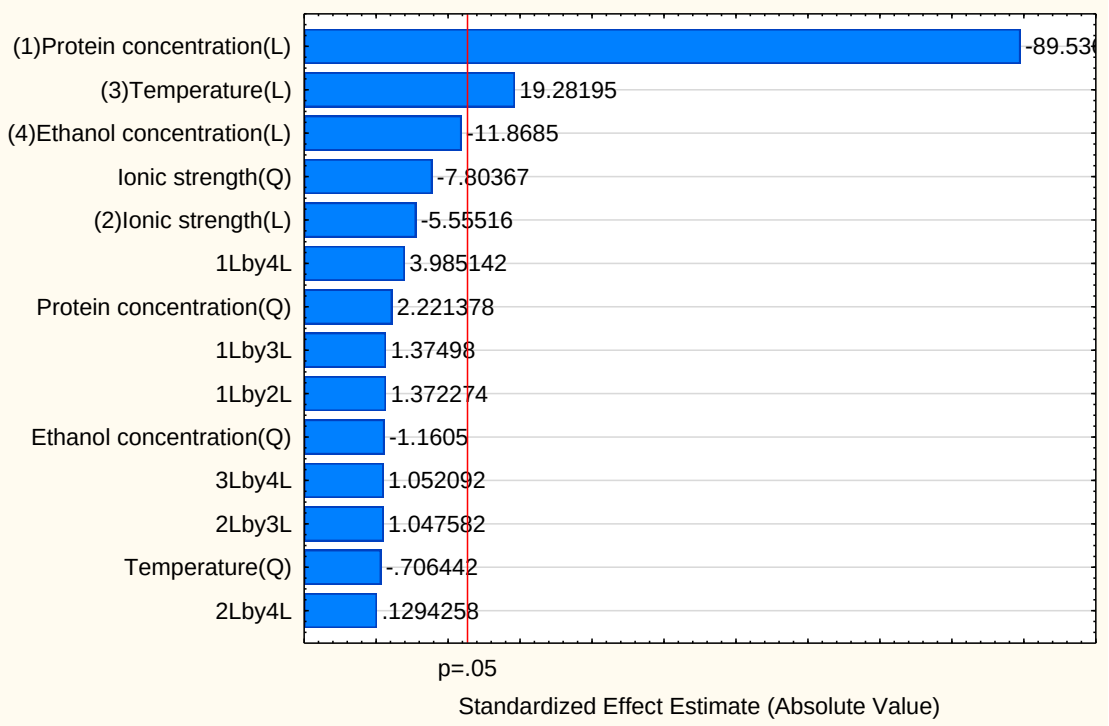


Figure 5:5: *Pareto chart of the standardized effects for diafiltration step two of ultrafiltration. Protein concentration and temperature had a statistically significant ($p < 0.05$) effect on permeate flux during this step of ultrafiltration. Ethanol concentration had a substantive significant effect on permeate flux during this step of ultrafiltration*

Table 5:7: *ANOVA table for diafiltration step two of ultrafiltration; indicating the p-value, Beta co-efficient, t value and 95% confidence intervals.*

FACTOR	EFFECT	STD. ERR. PURE ERR	t (1)	P	COEFF	STD. ERR. COEFF	-95% CNF LMT	+95% CNF LMT
Adjusted R^2 = 91.80%								
Mean/Interc	31.36	0.39	80.00	0.008	31.36	0.39	26.38	36.34
(1) Protein concentration (L)	-20.26	0.23	-89.54	0.007	-10.13	0.11	-11.57	-8.69
Protein concentration (Q)	0.59	0.27	2.22	0.269	0.29	0.13	-1.39	1.98
(2) Ionic strength (L)	-1.26	0.23	-5.56	0.113	-0.63	0.11	-2.07	0.81
Ionic strength (Q)	-2.07	0.27	-7.80	0.081	-1.04	0.13	-2.72	0.65
(3) Temperature(L)	4.36	0.23	19.28	0.033	2.18	0.11	0.74	3.62
Temperature(Q)	-0.19	0.27	-0.71	0.608	-0.09	0.13	-1.78	1.59
(4) Ethanol concentration (L)	-2.69	0.23	-11.87	0.054	-1.34	0.11	-2.78	0.09
Ethanol concentration (Q)	-0.31	0.27	-1.16	0.453	-0.15	0.13	-1.84	1.53
1L by 2L	0.38	0.28	1.37	0.401	0.19	0.14	-1.57	1.95
1L by 3L	0.38	0.28	1.38	0.400	0.19	0.14	-1.57	1.95
1L by 4L	1.10	0.28	3.99	0.157	0.55	0.14	-1.21	2.31
2L by 3L	0.29	0.28	1.05	0.485	0.15	0.14	-1.62	1.91
2L by 4L	0.04	0.28	0.13	0.918	0.02	0.14	-1.74	1.78
3L by 4L	0.29	0.28	1.05	0.484	0.15	0.14	-1.62	1.91

The analysis of variance (Table 5:7) of the permeate flux relating to the diafiltration step two for all experiments, revealed that protein concentration (t = -89.54, p= 0.0071, 95% CI[-11.51, -8.69]) and temperature (t = 19.28, p = 0.0330, 95% CI[0.74, 3.61]) had statistically significant impacts on permeate flux during this step of the UF of HSA. Ethanol concentration (t = -11.87, p = 0.0535, 95% CI[-2.78, 0.10]) and ionic strength (t = -5.56, p = 0.1134, 95% CI[-2.07, 0.81]) did not have a statistically significant impact on permeate flux during diafiltration step two. Similar to diafiltration step one, the substantive effect of ETOH concentration on permeate flux during this step of UF of HSA can be considered significant since the p-value (p = 0.0535) is close to $p \leq 0.05$ and this factor has a corresponding reducing effect on permeate flux of 2.78g/min to 0.10g/min within a certainty of 95%. An increase in the protein concentration of 1g/L decreased the permeate flux between 11.57g/min to 8.69g/min. An increase in the temperature of 1°C increased the permeate flux between 0.74g/min to 3.62g/min. The adjusted R^2 value (Table 5:7) suggested that at least 92.00% of the variability in permeate flux during diafiltration step two was accounted for by taking into consideration the effects of protein concentration and temperature. The validity of the above conclusions are supported by the linearity of the normal probability plot vs. raw residuals (data not shown). Note that the interactive effects between factors did not have significant impacts (Figure 5:5) on the permeate flux during this step.

Table 5:8: *Table showing the magnitude of the effects of the significant factors that influence permeate flux during diafiltration step two.*

Permeate Flux	**Protein Concentration (g/L)**	**Temperature (°C)**	**Ethanol Concentration (%)**
Co-efficient (β = +/-)	-10.13	2.18	-1.34
-95% (g/min)	-11.57	0.74	-2.78
+95% (g/min)	-8.69	3.62	0.09
-95% (ml/min)	-11.34	0.73	-2.73
+95% (ml/min)	-8.52	3.55	0.09
-95% (LMH)	-6.80	0.44	-1.64
+95% (LMH)	-5.11	2.13	0.06
L/h (30m2)	-204.09	13.13	-49.06
	-153.37	63.85	1.67

The magnitude of the effects of the significant factors that influence permeate flux during diafiltration step two are displayed in Table 5:8. Protein concentration and

temperature had a statistically significant impact on permeate flux during this step of UF of HSA. Ethanol concentration had a significant substantive effect on permeate flux. Protein concentration had an effect magnitude of -6.80LMH within the 95% confidence interval. This translates to a reduction of permeate flow rate of almost 205L/h, if 30m^2 of membrane area is used for the UF of HSA. Temperature has an effect magnitude of +2.13LMH within the 95% confidence interval, although the effect of temperature on permeate flux is smaller when compared to protein concentration. Ethanol concentration had a substantively significant impact on permeate flux during diafiltration step two; with an effect magnitude of -1.64LMH which is similar to the effect of temperature on permeate flux during this diafiltration step.

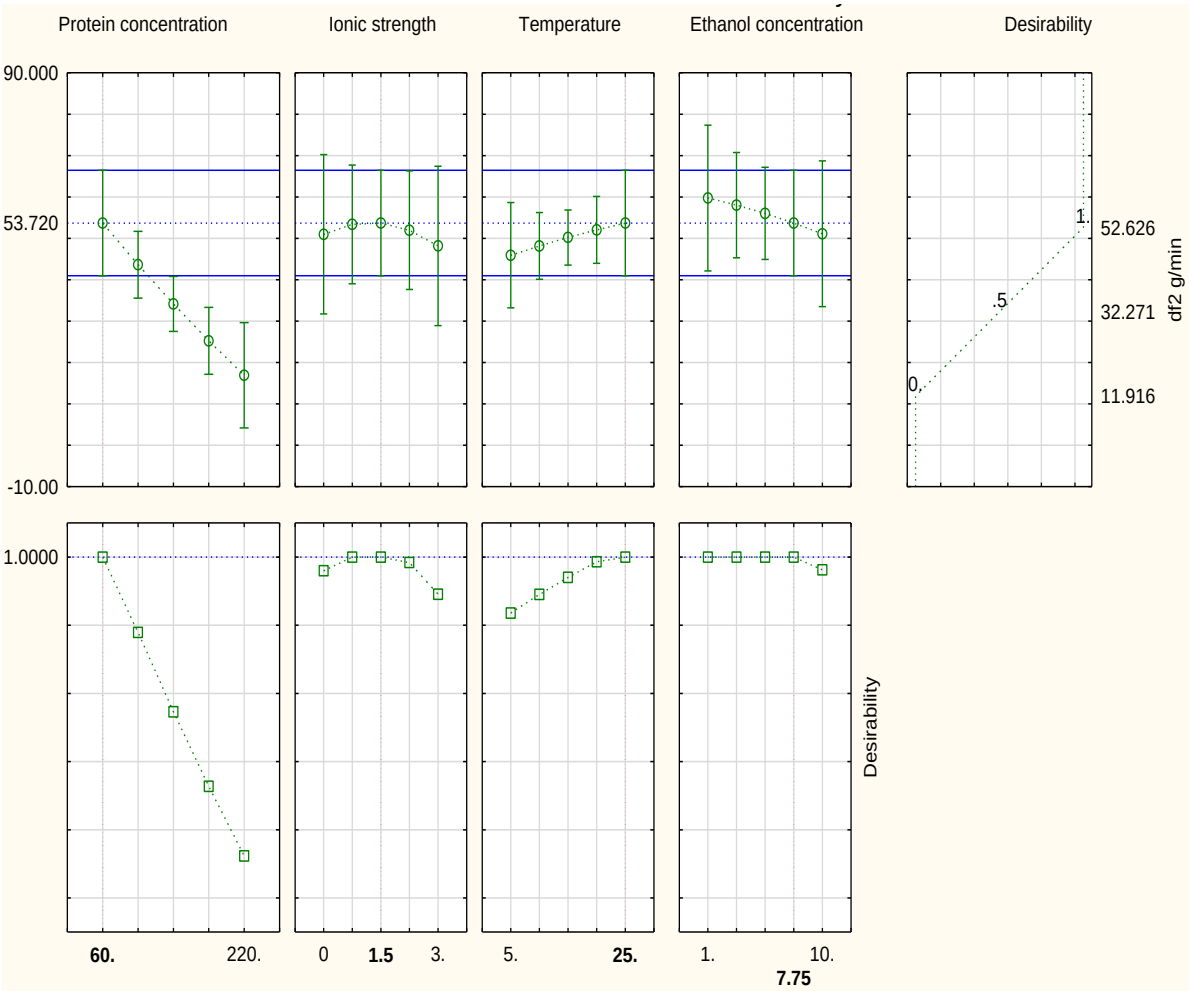


Figure 5:6: *Profile for predicted values and desirability for all factors for diafiltration step two of ultrafiltration. The desirability function is maximized to achieve highest permeate flux for all factors, during the ultrafiltration of human serum albumin, were 1.000 is most desirable response and 0.000 is least desirable response. The predicted value for protein concentration that achieves the higher permeate flux during diafiltration step two is 60g/L. The predicted value for temperature that achieves the highest permeate flux during diafiltration step two is 25°C.*

The formation of a gel boundary layer, coupled with CP causes permeate flux decline during UF of protein solutions until a relatively constant permeate flux (quasi-steady state permeate flux) is achieved, during steady state conditions (Marshall *et al.*, 1993). This phenomenon relating to permeate flux decline was observed in diafiltration step two for all experiments. The profile for predicted values and desirability (Figure 5:6) for diafiltration step two showed that permeate flux clearly decreased as protein concentration increased, with desired value for protein concentration during diafiltration step two equal to 60g/L. This value is similar to that obtained for diafiltration step one; the desired value for maximum permeate flux is dependent on CP and MF which in turn is dependent on the protein concentration at the start of UF of HSA, therefore maximum permeate flux during this process is always achieved at the lowest protein concentration. Permeate flux increased with increase in temperature during this step of UF of HSA, with the optimum temperature for maximum flux specified at 25°C. Similar to diafiltration step one, a temperature of 20°C produced a desirability value close to 1.000 and will also be considered when determining the optimum temperature set point for this study, for reasons stated previously. The predicted values for diafiltration step two with respect to protein concentration and temperature were identical to those determined for diafiltration step one. Ethanol concentration had a substantive significance on permeate flux during diafiltration step two, with decrease in permeate flux with increase in ETOH concentration noted within the selected range of this factor. The profile for predicted values and desirability for diafiltration step two declared a desired value for ETOH concentration within the range 0% (v/v) to 7.5% (v/v). Ethanol concentration shows a turning point towards lower permeate flux at 7.5% (v/v). The profile for predicted value and desirability for ionic strength was not be analysed since the impact of this factor on flux was considered insignificant ($p > 0.05$).

5.4.4 STATISTICAL ANALYSIS OF DIAFILTRATION STEP THREE

Diafiltration step three is the fourth step of the UF of HSA and the third step of CVD; immediately following diafiltration step two. The end of diafiltration step three was defined as the collection of permeate volume equal to three times the bed volume of the bulk solution (dissolved Fraction V paste) which was determined at the end of the first concentration step. The average mass flow rates (g/min) recorded during diafiltration step three for all experiments are presented in Table 5:1.

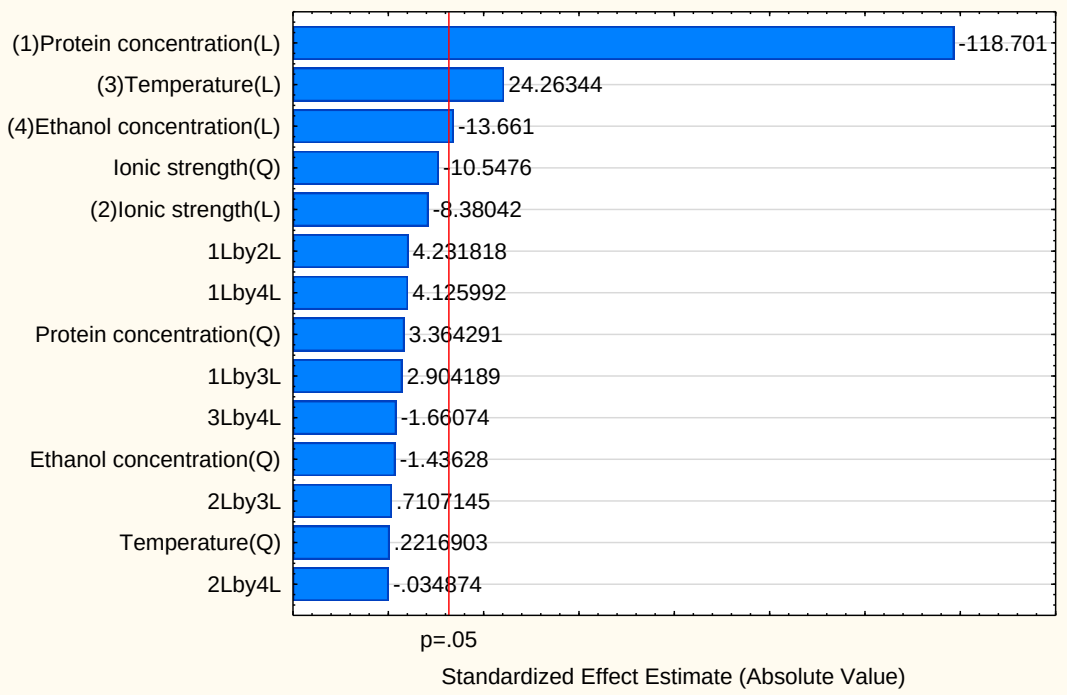


Figure 5:7: *Pareto chart of the standardized effects for diafiltration step three of ultrafiltration. Protein concentration, temperature and ethanol concentration had a statistically significant ($p < 0.05$) effect on permeate flux during this step of ultrafiltration.*

***Table 5:9**: ANOVA table for diafiltration step three of ultrafiltration; indicating the p-value, Beta co-efficient, t value and 95% confidence intervals.*

FACTOR	EFFECT	STD. ERR. PURE ERR	t (1)	P	COEFF	STD. ERR. COEFF	-95% CNF LMT	+95% CNF LMT
Adjusted R^2 = 91.90%								
Mean/Interc	31.46	0.29	107.00	0.006	31.46	0.29	27.72	35.19
(1) Protein concentration (L)	-20.15	0.17	-118.70	0.005	-10.07	0.08	-11.15	-9.00
Protein concentration (Q)	0.67	0.20	3.36	0.184	0.33	0.10	-0.93	1.60
(2) Ionic strength (L)	-1.42	0.17	-8.38	0.076	-0.71	0.08	-1.79	0.37
Ionic strength (Q)	-2.10	0.20	-10.55	0.060	-1.05	0.10	-2.31	0.21
(3) Temperature(L)	4.12	0.17	24.26	0.026	2.06	0.08	0.98	3.14
Temperature(Q)	0.04	0.20	0.22	0.861	0.02	0.10	-1.24	1.29
(4) Ethanol concentration (L)	-2.32	0.17	-13.66	0.047	-1.16	0.08	-2.24	-0.08
Ethanol concentration (Q)	-0.29	0.20	-1.44	0.387	-0.14	0.10	-1.41	1.12
1L by 2L	0.88	0.21	4.23	0.148	0.44	0.10	-0.88	1.76
1L by 3L	0.60	0.21	2.90	0.211	0.30	0.10	-1.02	1.62
1L by 4L	0.86	0.21	4.13	0.151	0.43	0.10	-0.89	1.75
2L by 3L	0.15	0.21	0.71	0.607	0.07	0.10	-1.25	1.39
2L by 4L	-0.01	0.21	-0.04	0.978	0.00	0.10	-1.32	1.32
3L by 4L	-0.35	0.21	-1.66	0.345	-0.17	0.10	-1.49	1.15

The analysis of variance (Table 5:9) of the permeate flux relating to the diafiltration step three for all experiments, revealed that protein concentration (t = -118.70, p= 0.0054, 95% CI[-11.15, -9.00]), temperature (t = 19.28, p = 0.0262, 95% CI[0.98, 3.14]) and ETOH concentration (t = -13.66, p = 0.0465, 95% CI[-2.24, -0.08]) had statistically significant impacts on permeate flux during this step of the UF of HSA. Ionic strength (t = -8.38, p = 0.0756, 95% CI[-1.79, 0.37]) does not have a statistically significant impact on permeate flux during diafiltration step three. An increase in the protein concentration of 1g/L results in a decrease of the permeate flux between 11.15g/min to 9.00g/min. An increase in the temperature of 1°C results in an increase of the permeate flux between 0.98g/min to 3.14g/min. An increase in the ETOH concentration of 1% results in a decrease of the permeate flux between 2.24g/min to 0.08g/min. The adjusted R^2 value (Table 5:9) suggested that at least 92.00% of the variability in permeate flux during diafiltration step three is accounted for by taking into consideration the effects of protein concentration, temperature and ETOH concentration. The validity of the above conclusions are supported by the linearity of the normal probability plot vs. raw residuals (data not shown). Note that the interactive effects (Figure 5:7) between factors does not have a significant impact on permeate flux during this step.

Table 5:10: *Table showing the magnitude of the effects of the significant factors that influence permeate flux during diafiltration step three.*

Permeate Flux	Protein Concentration (g/L)	Temperature (°C)	Ethanol Concentration (%)
Co-efficient (β = +/-)	-10.07	2.06	-1.16
-95% (g/min)	-11.15	0.98	-2.24
+95% (g/min)	-9.00	3.14	-0.08
-95% (ml/min)	-10.93	0.96	-2.19
+95% (ml/min)	-8.82	3.07	-0.08
-95% (LMH)	-6.56	0.58	-1.32
+95% (LMH)	-5.29	1.84	-0.05
L/h (30m^2)	-196.73	17.30	-39.47
	-158.69	55.35	-1.43

The magnitude of the effects of the significant factors that influence permeate flux during diafiltration step three are displayed in Table 5:10. Protein concentration, temperature and ETOH concentration had a statistically significant impact on permeate flux during this step of UF of HSA. Protein concentration had an effect magnitude of -6.56LMH within the 95% confidence interval. This translates to a reduction of permeate flow rate of almost 197L/H, if 30m^2 of membrane area is used for the UF of HSA. Temperature has an effect magnitude of +1.84LMH within the 95% confidence interval, although the effect of temperature on permeate flux is smaller when compared to protein concentration. Ethanol concentration had an effect magnitude of -1.32LMH which is similar to the effect of temperature on permeate flux during this diafiltration step.

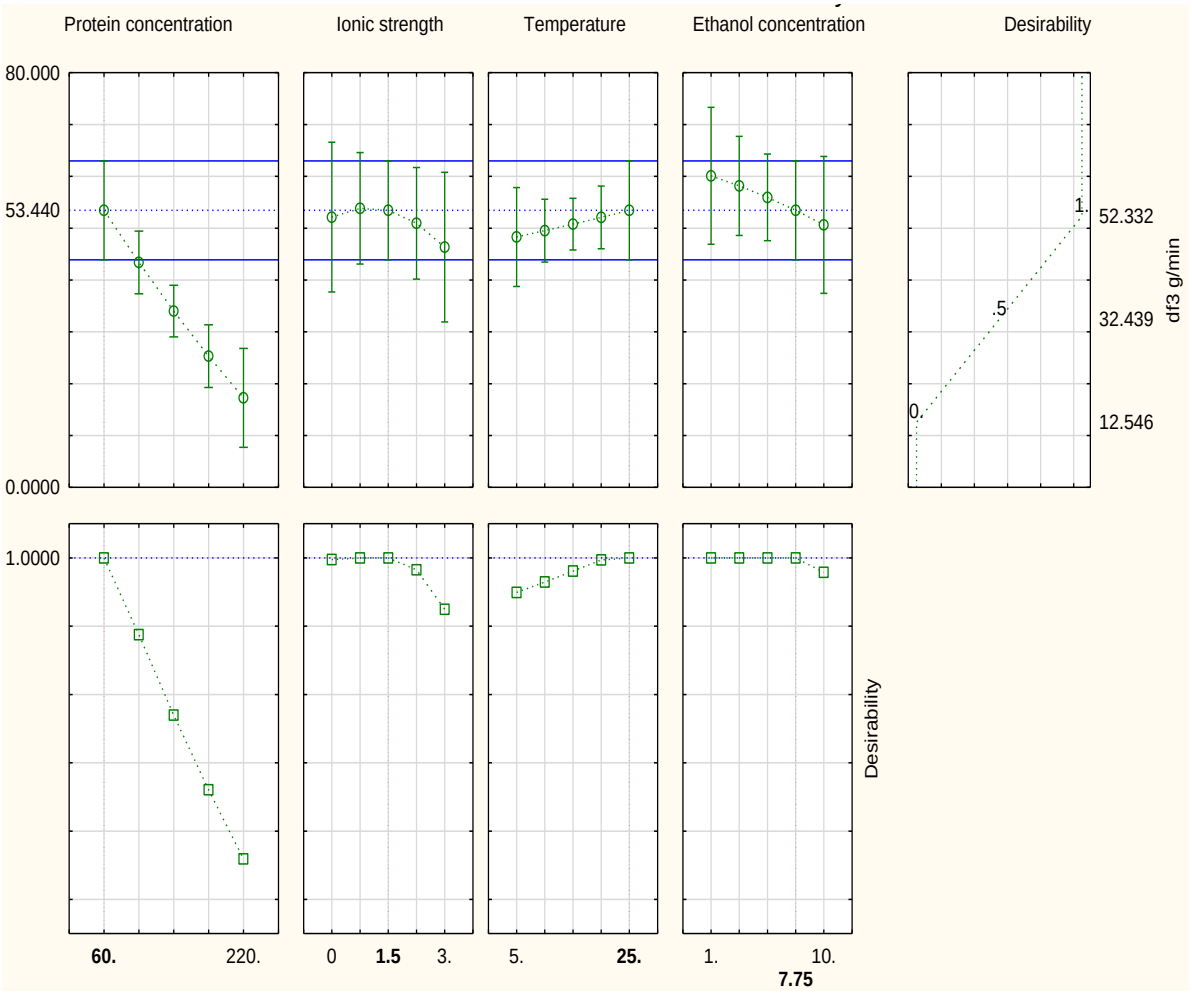


Figure 5:8: *Profile for predicted values and desirability for all factors for diafiltration step three of ultrafiltration. The desirability function is maximized to achieve highest permeate flux for all factors, during the ultrafiltration of human serum albumin, were 1.000 is most desirable response and 0.000 is least desirable response. The predicted value for protein concentration that achieves the higher permeate flux during diafiltration step three is 60g/L; for temperature the highest flux is achieved at 25°C and for ethanol concentration the highest flux is achieved within the range 1% to 7.75%.*

The quasi-steady state permeate flux noted in diafiltration step two, continues in diafiltration step three, for all experiments (Marshall *et al.*,1993). The statistical analysis of diafiltration step three revealed that protein concentration, ETOH concentration and temperature had significant impacts (Figure 5:7) on permeate flux during this step of UF of HSA. The profile for predicted values and desirability (Figure 5:8) showed that permeate flux clearly decreases as protein concentration increases; with desired value for protein concentration during diafiltration step three equal to 60g/L. This value is similar to diafiltration steps one and two; similar conclusions can be derived based on the CP and MF effects due to protein concentration. Permeate flux increases with increase in temperature during this step of UF of HSA; with the optimum

temperature for maximum flux specified at 25°C. Similar to diafiltration steps one and two, a temperature of 20°C produced a desirability value close to 1.000 and will also be considered when determining the optimum temperature set point for this study, for reasons stated previously. Figure 5:8 clearly indicated that optimum ETOH concentration lies within the range 0% (v/v) to 7.5% (v/v); with a distinct turning point towards lower permeate flux at 7.75% (v/v) ETOH concentration, which is similar to diafiltration step two. The profile for predicted value and desirability for ionic strength will not be analysed since the impact of this factor on flux was considered insignificant ($p > 0.05$).

5.4.5 STATISTICAL ANALYSIS OF DIAFILTRATION STEP FOUR

Diafiltration step four is the fifth step of the UF of HSA and the fourth cycle of CVD; immediately following diafiltration step three. The end of diafiltration step four was defined as the collection of permeate volume equal to four times the bed volume of the bulk solution (dissolved Fraction V paste) which was determined at the end of the first concentration step. The average mass flow rates (g/min) recorded during diafiltration step four for all experiments are presented in Table 5:1.

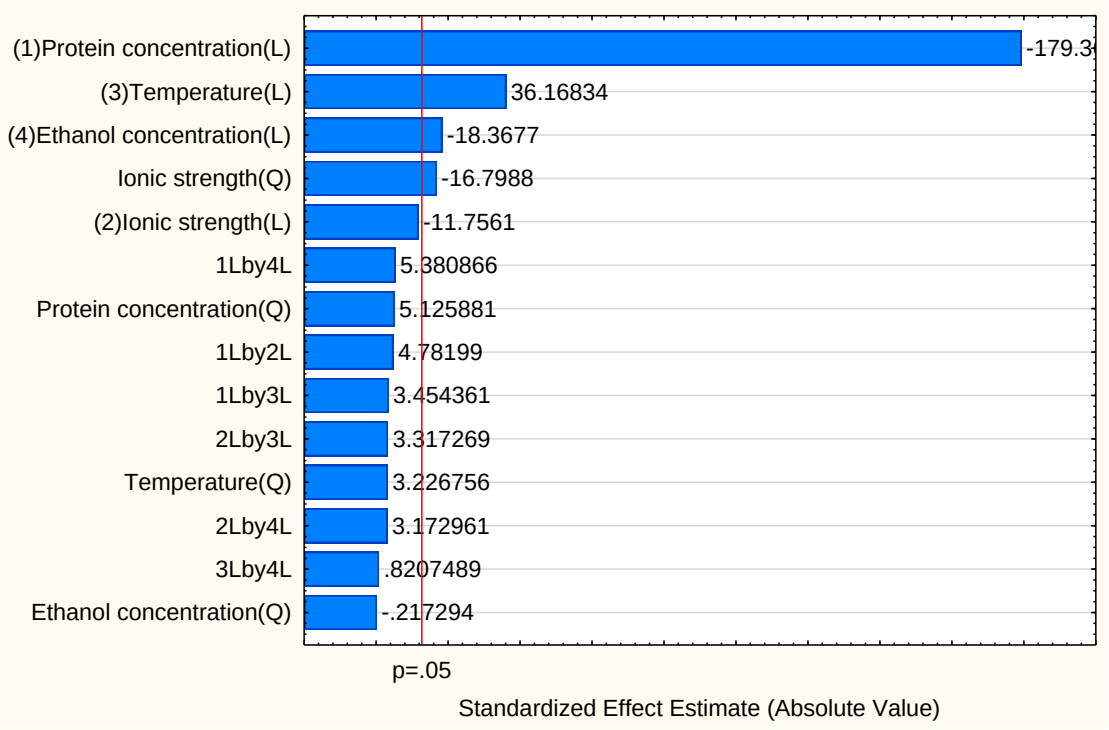


Figure 5:9: *Pareto chart of the standardized effects for diafiltration step four of ultrafiltration. Protein concentration, temperature, ethanol concentration and ionic strength had a statistically significant ($p < 0.05$) effect on permeate flux during this step of ultrafiltration.*

Table 5:11: *ANOVA table for diafiltration step four of ultrafiltration; indicating the p-value, Beta co-efficient, t value and 95% confidence intervals.*

FACTOR	EFFECT	STD. ERR. PURE ERR	t (1)	P	COEFF	STD. ERR. COEFF	-95% CNF LMT	+95% CNF LMT
Adjusted R^2 = 90.43%								
Mean/Interc	31.56	0.20	161.00	0.004	31.56	0.20	29.07	34.05
(1) Protein concentration (L)	-20.30	0.11	-179.36	0.004	-10.15	0.57	-10.87	-9.43
Protein concentration (Q)	0.68	0.13	5.13	0.123	0.36	0.07	-0.50	1.18
(2) Ionic strength (L)	-1.33	0.11	-11.76	0.054	-0.67	0.06	-1.38	0.05
Ionic strength (Q)	-2.23	0.13	-16.80	0.038	-1.11	0.07	-1.96	-0.27
(3) Temperature(L)	4.09	0.11	36.17	0.018	2.05	0.06	1.33	2.77
Temperature(Q)	0.43	0.13	3.23	0.191	0.21	0.07	-0.63	1.06
(4) Ethanol concentration (L)	-2.08	0.11	-18.37	0.035	-1.04	0.06	-1.76	-0.32
Ethanol concentration (Q)	-0.03	0.13	-0.22	0.864	-0.01	0.07	-0.86	0.83
1L by 2L	0.66	0.13	4.78	0.131	0.33	0.07	-0.55	1.21
1L by 3L	0.48	0.13	3.45	0.179	0.24	0.07	-0.64	1.12
1L by 4L	0.75	0.13	5.38	0.117	0.37	0.07	-0.51	1.25
2L by 3L	0.46	0.13	3.32	0.186	0.23	0.07	-0.65	1.11
2L by 4L	0.44	0.13	3.17	0.194	0.22	0.07	-0.66	1.10
3L by 4L	0.11	0.13	0.82	0.562	0.06	0.07	-0.82	0.94

The analysis of variance (Table 5:11) of the permeate flux relating to the diafiltration step four for all experiments, revealed that protein concentration (t = -179.36, p= 0.0035, 95% CI[-10.87, -9.43]), temperature (t = 36.17, p = 0.0175, 95% CI[1.33, 2.77]) and ETOH concentration (t = -18.37, p = 0.0346, 95% CI[-1.76, -0.32]) had statistically significant impacts on permeate flux during this step of the UF of HSA. Ionic strength (t = -11.76, p = 0.0540, 95% CI[-1.38, 0.05]) does not have a statistically significant impact on permeate flux during diafiltration step four . However the substantive effect of ionic strength on permeate flux during this step of UF of HSA can be considered significant since the p-value (p = 0.0540) is close to p-value ≤ 0.05 and this factor has a corresponding reducing effect on permeate flux of 1.38g/min to 0.05g/min within a certainty of 95%. An increase in the protein concentration of 1g/L resulted in a decrease of the permeate flux between 10.87g/min to 9.43g/min. An increase in the temperature of 1°C resulted in an increase of the permeate flux between 1.33g/min to 2.77g/min. An increase in the ETOH concentration of 1% resulted in a decrease of the permeate flux between 1.76g/min to 0.05g/min. The adjusted R^2 value (Table 5:11) suggested that at least 90.43% of the variability in permeate flux during diafiltration step four is accounted for by taking into consideration the effects of protein concentration, temperature and ETOH concentration. The validity of the above conclusions are

supported by the linearity of the normal probability plot vs. raw residuals (data not shown). Note that the interactive effects (Figure 5:9) between factors does not have a significant impact on permeate flux during this step.

Table 5:12: *Table showing the magnitude of the effects of the significant factors that influence permeate flux during diafiltration step four.*

Permeate Flux	Protein Concentration (g/L)	Temperature (°C)	Ethanol Concentration (%)
Co-efficient (β = +/-)	-10.15	2.05	-1.04
-95% (g/min)	-10.87	1.33	-1.76
+95% (g/min)	-9.43	2.77	-0.32
-95% (ml/min)	-10.65	1.30	-1.72
+95% (ml/min)	-9.24	2.71	-0.31
-95% (LMH)	-6.39	0.78	-1.03
+95% (LMH)	-5.54	1.63	-0.19
L/h (30m^2)	-191.70	23.42	-31.01
	-166.34	48.78	-5.65

The magnitude of the effects of the significant factors that influence permeate flux during diafiltration step four are displayed in Table 5:12. Protein concentration, temperature and ETOH concentration had a statistically significant impact (Figure 5:9) on permeate flux during this step of UF of HSA. Protein concentration had an effect magnitude of -6.39LMH within the 95% confidence interval. This translates to a reduction of permeate flow rate of almost 191L/H, if 30m^2 of membrane area is used for the UF of HSA. Temperature has an effect magnitude of +1.63LMH within the 95% confidence interval, although the effect of temperature on permeate flux is smaller when compared to protein concentration. Ethanol concentration had an effect magnitude of -1.03LMH which is similar to the effect of temperature on permeate flux during this diafiltration step.

The CP and MF phenomenon during UF of HSA continues to effect permeate flux during diafiltration step four such that the flux was noted to be steady, with minimal increase in flux noted. This was noted for diafiltration step four, for all experiments completed during this study. The statistical analysis of diafiltration step four revealed that protein concentration, ETOH concentration and temperature had significant impacts on permeate flux during this step of UF of HSA.

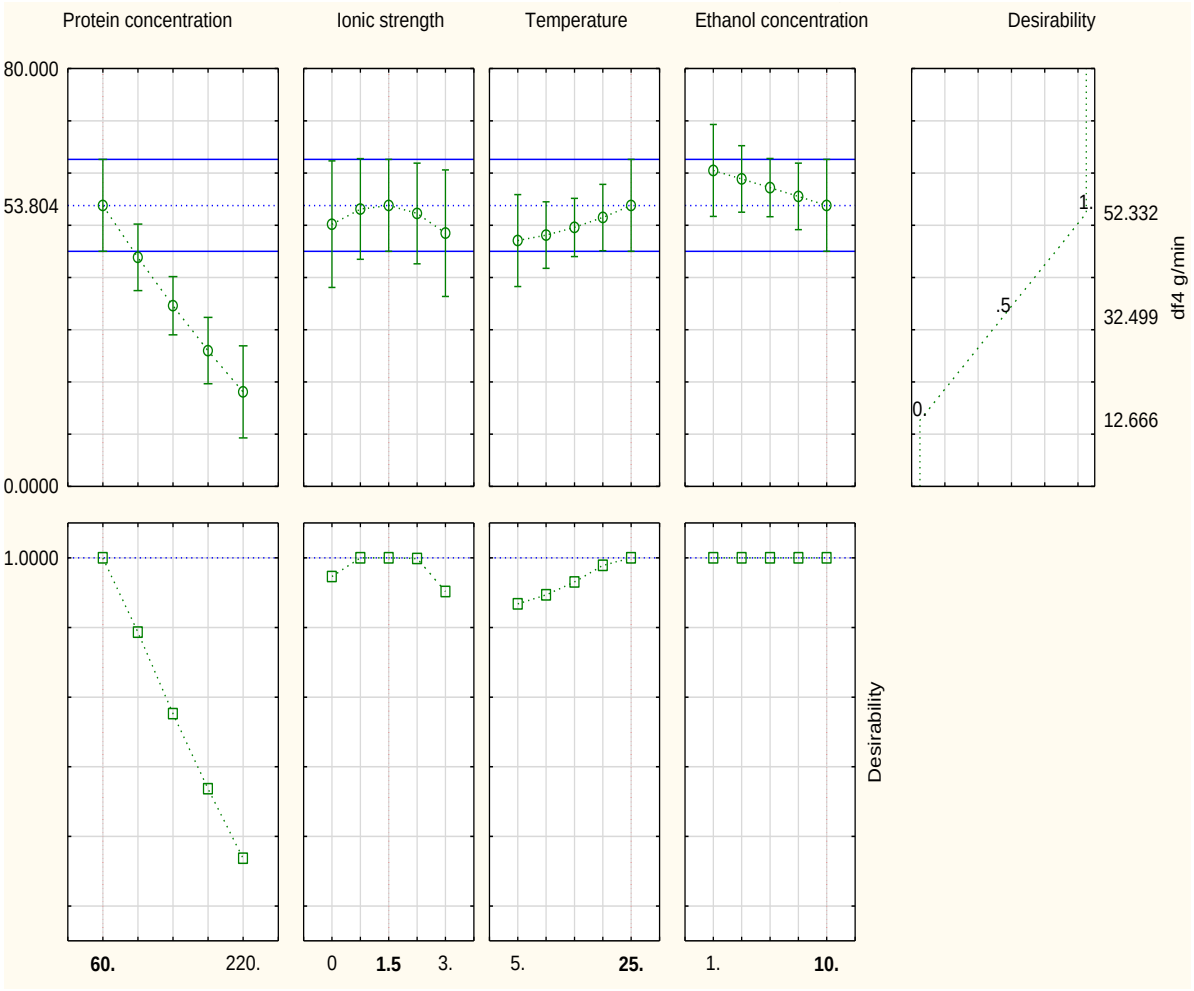


Figure 5:10: *Profile for predicted values and desirability for all factors for diafiltration step four of ultrafiltration. The desirability function is maximized to achieve highest permeate flux for all factors, during the ultrafiltration of human serum albumin, were 1.000 is most desirable response and 0.000 is least desirable response. The predicted value for protein concentration that achieves the higher permeate flux during diafiltration step four is 60g/L; for temperature the highest flux is achieved at 25°C and for ethanol concentration the highest flux is achieved within the range 1% to 10.0%. A turning point between permeate flux increase/decrease occurs at 1.5M ionic strength.*

The profile for predicted values and desirability (Figure 5:10) for diafiltration step four showed that permeate flux clearly decreases as protein concentration increases; with desired value for protein concentration during diafiltration step four equal to 60g/L. Permeate flux increased with increase in temperature during this step of UF of HSA; with the optimum temperature for maximum flux specified at 25°C. Similar to diafiltration steps one, two and three, another predicted value for temperature is 20°C and this could be used as an alternate temperature level for this factor, for reasons stated previously. The profile for predicted values and desirability (Figure 5:10) clearly indicated that optimum ETOH concentration lies within the range 0% (v/v) to 10.0%

(v/v); with no distinct turning point towards lower permeate flux within this range. Since ionic strength had a minimal, substantive effect on permeate flux during this step of UF of HSA, the ionic strength predicted value lies within a range of 0.75M and 2.25M, with definite turning points showing a decrease of flux at values lower or higher than this stipulated range.

5.4.6 STATISTICAL ANALYSIS OF DIAFILTRATION STEP FIVE

Diafiltration step five is the fifth step of the UF of HSA and the fourth cycle of CVD. Diafiltration step five follows immediately after diafiltration step four. The end of diafiltration step five was defined as the collection of permeate volume equal to five times the bed volume of the bulk solution (dissolved Fraction V paste) which was determined at the end of the first concentration step. The mass flow rates (g/min) during diafiltration step five for all experiments are presented in Table 5:1.

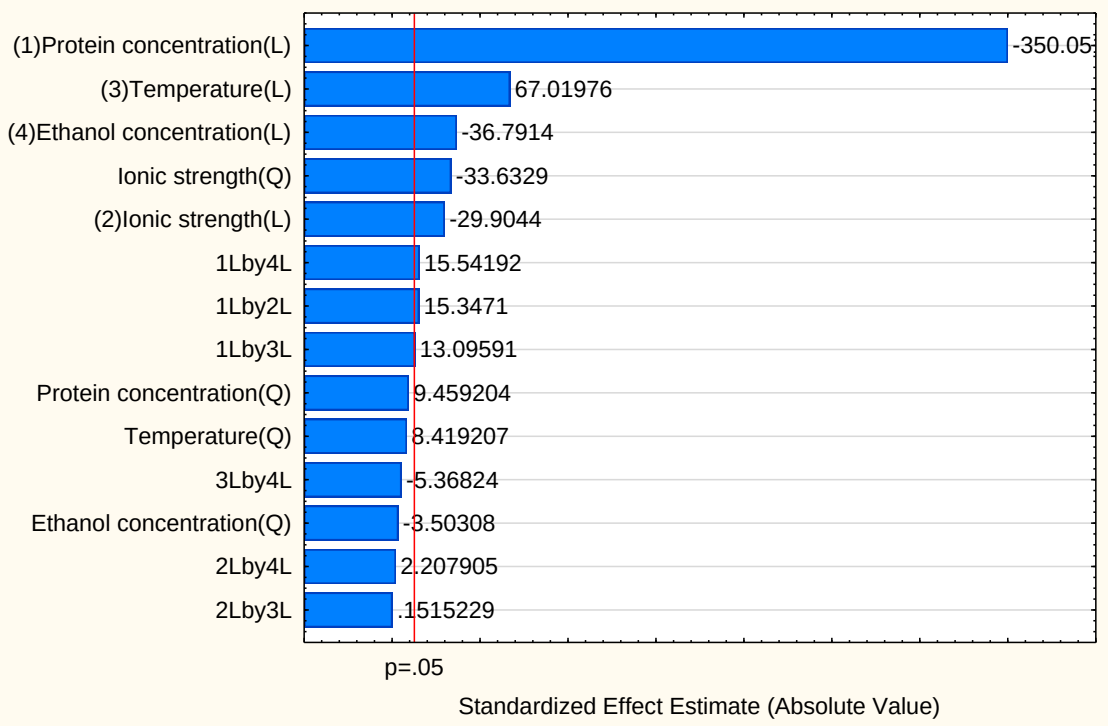


Figure 5:11: *Pareto chart of the standardized effects for diafiltration step five of ultrafiltration. All factors had a statistically significant ($p < 0.05$) effect on permeate flux during this step of ultrafiltration. Further, protein concentration has a significant interactive effect with all other factors.*

Table 5:13: *ANOVA table for diafiltration step five of ultrafiltration; indicating the p-value, Beta co-efficient, t value and 95% confidence intervals.*

FACTOR	EFFECT	STD. ERR. PURE ERR	t (1)	P	COEFF	STD. ERR. COEFF	-95% CNF LMT	+95% CNF LMT
Adjusted R^2 = 89.56%								
Mean/Interc	34.65	0.10	323.00	0.002	31.65	0.10	30.41	32.90
(1) Protein concentration (L)	-19.81	0.06	-350.05	0.002	-9.90	0.03	-10.26	-9.54
Protein concentration (Q)	0.63	0.07	9.46	0.067	0.31	0.03	-0.11	0.74
(2) Ionic strength (L)	-1.69	0.06	-29.90	0.021	-0.85	0.03	-1.21	-0.49
Ionic strength (Q)	-2.23	0.07	-33.63	0.019	-1.12	0.03	-1.54	-0.69
(3) Temperature(L)	3.79	0.06	67.02	0.009	1.90	0.03	1.54	2.26
Temperature(Q)	0.56	0.07	8.42	0.075	0.28	0.03	-0.14	0.70
(4) Ethanol concentration (L)	-2.08	0.06	-36.79	0.017	-1.04	0.03	-1.40	-0.68
Ethanol concentration (Q)	-0.23	0.07	-3.50	0.177	-0.12	0.03	-0.54	0.31
1L by 2L	1.06	0.07	15.35	0.041	0.53	0.03	0.09	0.97
1L by 3L	0.91	0.07	13.10	0.049	0.45	0.03	0.01	0.89
1L by 4L	1.08	0.07	15.54	0.041	0.54	0.03	0.10	0.98
2L by 3L	0.01	0.07	0.15	0.904	0.01	0.03	-0.44	0.45
2L by 4L	0.15	0.07	2.21	0.271	0.08	0.03	-0.36	0.52
3L by 4L	-0.37	0.07	-5.37	0.117	-0.19	0.03	-0.63	0.25

The analysis of variance (Table 5:13) of the permeate flux relating to the diafiltration step five for all experiments, reveals that protein concentration (t = -350.05, p= 0.0018, 95% CI[-10.26, -9.54]), temperature (t = 67.02, p = 0.0094, 95% CI[1.54, 2.26]), ETOH concentration (t = -36.80, p = 0.0172, 95% CI[-1.40, -0.68]) and ionic strength concentration (t = -29.90, p = 0.0213, 95% CI[-1.21, -0.49]) have a statistically significant impact on permeate flux during this step of the UF of HSA. Several interactive effects are noted but these will be discussed later. An increase in the protein concentration of 1g/L resulted in a decrease of the permeate flux between -10.26g/min (-5.61LMH) to -9.54g/min (-6.03LMH). An increase in the temperature of 1°C resulted in an increase of the permeate flux between 1.54g/min (0.90LMH) to 2.26g/min (1.333LMH). An increase in the ETOH concentration of 1% resulted in a decrease of the permeate flux between -1.40g/min (-0.82LMH) to -0.68g/min (-0.40LMH). An increase in the ionic strength by 1M resulted in a decrease of the permeate flux between -1.21g/min (-0.71LMH) to -0.49g/min (-0.29MH). The adjusted R^2 value (Table 5:13) suggests that at least 90.43% of the variability in permeate flux during diafiltration step five is accounted for by taking into consideration the effects of protein concentration, temperature, ionic strength and ETOH concentration. The validity of the above conclusions are supported by the linearity of the normal probability plot vs. raw

residuals (data not shown). During diafiltration step five several interactive effects are considered significant (Figure 5:11). These significant interactive effects are between protein concentration and ionic strength (t = -15.35, p = 0.0414, 95% CI[0.0915, -0.9720]); protein concentration and ETOH concentration (t = 13.096, p = 0.0485, 95% CI[0.0135, 0.8940]) and protein concentration and temperature ((t = 15.542, p = 0.0409, 95% CI[0.0983, -0.9788]).

Table 5:14: *Table showing the magnitude of the effects of the significant factors that influence permeate flux during diafiltration step five.*

Permeate Flux	Protein Concentration (g/L)	Ionic Strength (M)	Temperature (°C)	Ethanol Concentration (%)	1L x 2L	1L x 3L	1L x 4L
Co-efficient (β = +/-)	-9.90	-0.85	1.90	1.04	0.53	0.45	0.54
-95% (g/min)	-10.26	-1.21	1.54	-1.40	0.09	0.01	0.10
+95% (g/min)	-9.54	-0.49	2.26	-0.68	0.97	0.89	0.98
-95% (ml/min)	-10.06	-1.18	1.51	-1.37	0.09	0.01	0.10
+95% (ml/min)	-9.35	-0.48	2.21	-0.67	0.95	0.88	0.96
-95% (LMH)	6.03	0.71	0.90	0.82	0.05	0.01	0.06
+95% (LMH)	5.61	0.29	1.33	0.40	0.57	0.53	0.58
L/h (30m^2)	181.03	21.27	27.10	24.70	1.61	0.24	1.73
	168.35	8.58	39.79	12.02	17.15	15.77	17.27

The magnitude of the effects of significant factors is displayed in Table 5:14. All factors had a significant effect (Figure 5:11) on permeate flux during diafiltration step five. Further, the interactive effects between protein concentration and ionic strength, temperature and ETOH concentration was also considered statistically significant (Figure 5.11). However, it is important to note that although all factors and interactive effects are considered statistically significant, only protein concentration has an effect magnitude of greater than 5LMH on permeate flux during this step of UF of HSA. All other factors, including the interactive effects have an effect magnitude of less than 1.5LMH, within the 95% confidence interval determined for that factor or interactive factor.

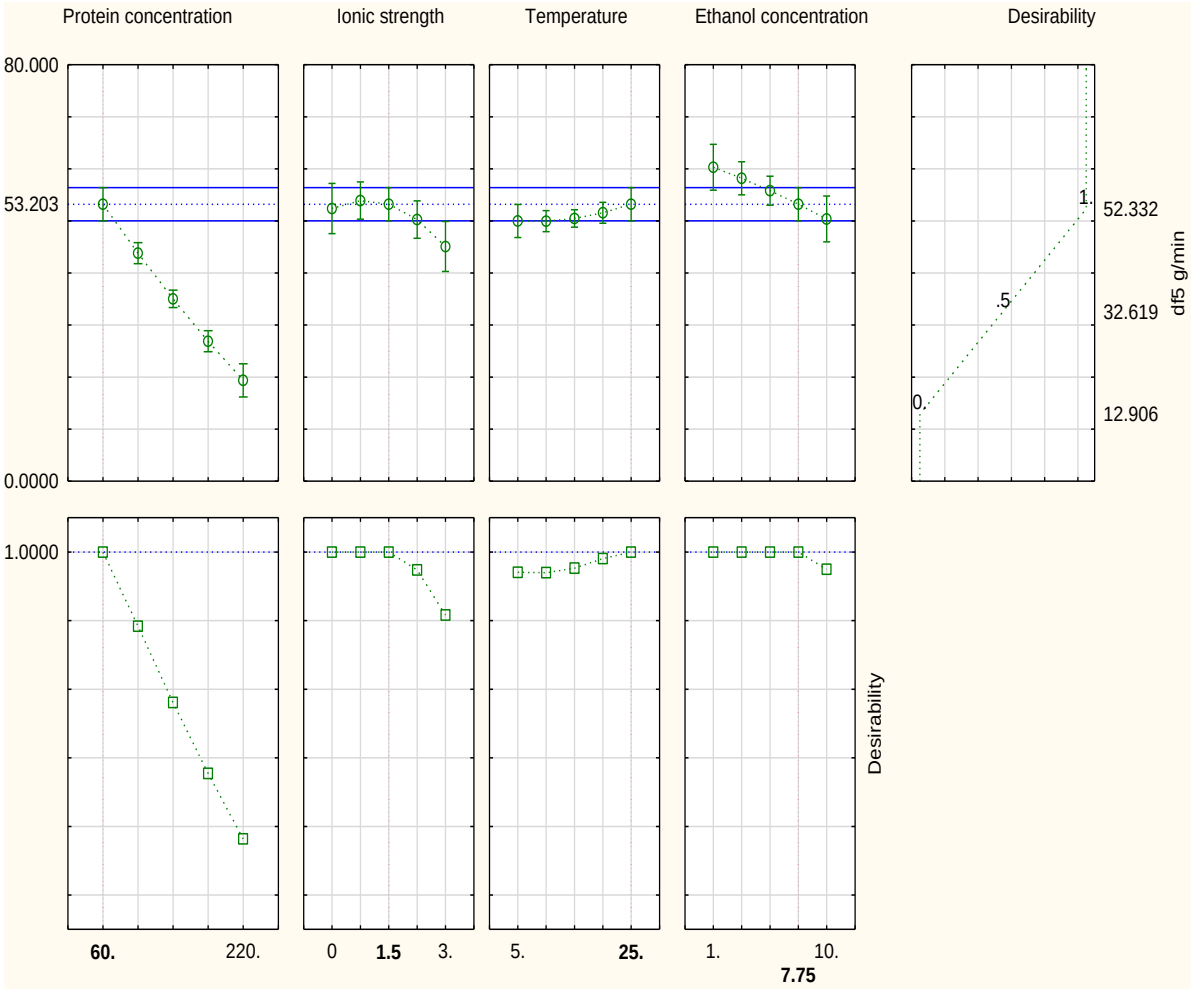


Figure 5:12: *Profile for predicted values and desirability for all factors for diafiltration step five of ultrafiltration. The desirability function is maximized to achieve highest permeate flux for all factors, during the ultrafiltration of human serum albumin, were 1.000 is most desirable response and 0.000 is least desirable response. The predicted value for protein concentration that achieves the higher permeate flux during diafiltration step five is 60g/L; for the highest flux is achieved at 25°C and for ethanol concentration the highest flux is achieved within the range 1% to 7.75%. The desirable value for ionic strength lies within the range 0M to 1.5M.*

The permeate flux during diafiltration step five is described as quasi-steady state and similar to the permeate flux trend noted in diafiltration steps three and four. This permeate flux trend is consistent with theory suggested by other authors (Marshall *et al.*, 1983). The statistical analysis of diafiltration step five revealed that all factors had significant impact (Figure 5:11) on permeate flux during this step of UF of HSA. The profile for predicted values and desirability (Figure 5:12) for diafiltration step five showed that permeate flux decreased as protein concentration increased; with desired value for protein concentration during diafiltration step five equal to 60g/L. Permeate flux increased with increase in temperature during this step of UF of HSA; with the

optimum temperature for maximum flux specified at 25°C. Similarly to diafiltration steps three and four, the predicted value for temperature is also very close to 20°C and this value could be used as an alternate temperature level for this factor. During diafiltration step five, ETOH concentration decreased permeate flux when used in concentrations greater than 7.75%. Therefore the optimum ETOH concentration for this step of diafiltration is between the range 0% to 7.75%. Lastly, an increase in ionic strength resulted in a decrease of permeate flux. The optimum range for ionic strength lies between 0M and 0.75M.

5.4.7 STATISTICAL ANALYSIS OF THE SECOND CONCENTRATION STEP

During the second concentration step, the diafiltered HSA solution is concentrated to a final protein concentration of approximately 220g/L.

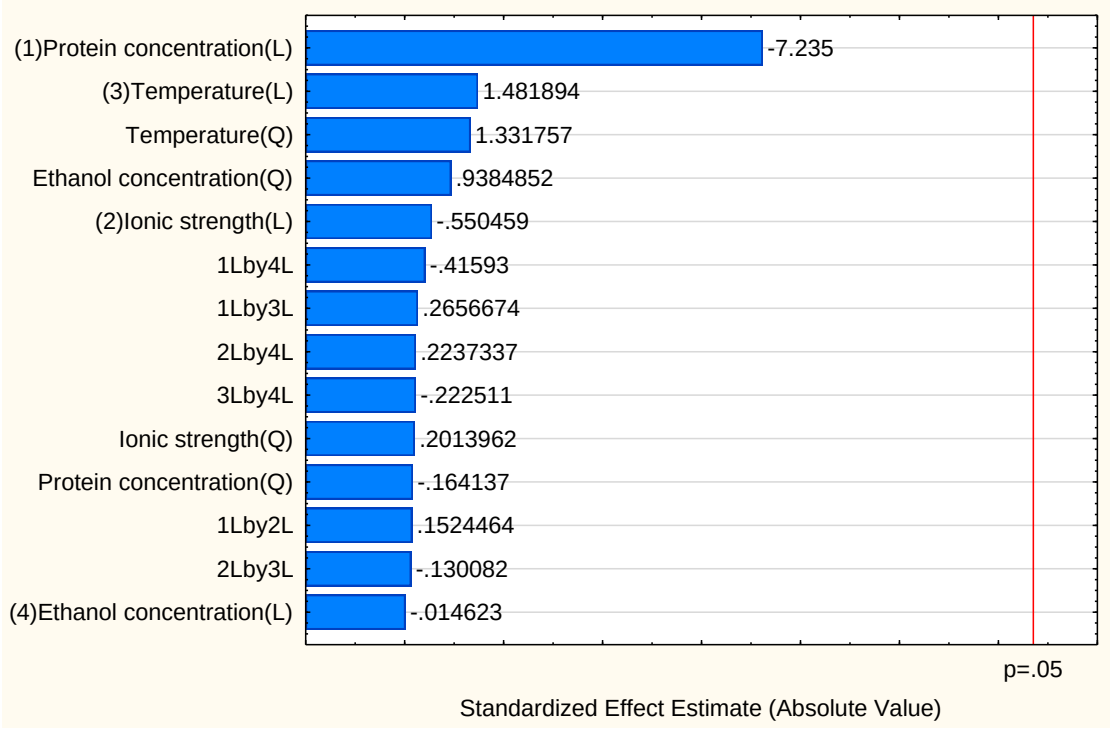


Figure 5:13: *Pareto chart of the standardized effects for the second concentration step of ultrafiltration. No factors had a statistically significant ($p < 0.05$) effect on permeate flux during this step of ultrafiltration.*

Table 5:15: *ANOVA table for second concentration step of ultrafiltration; indicating the p-value, Beta co-efficient, t value and 95% confidence intervals.*

FACTOR	EFFECT	STD. ERR. PURE ERR	t (1)	P	COEFF	STD. ERR. COEFF	-95% CNF LMT	+95% CNF LMT
Adjusted R^2 = 75.00%								
Mean/Interc	23.65	4.05	5.84	0.108	23.65	4.05	-27.77	75.07
(1) Protein concentration (L)	-16.90	2.34	-7.24	0.087	-8.45	1.17	-23.30	6.39
Protein concentration (Q)	-0.45	2.74	-0.16	0.896	-0.22	1.37	-17.63	17.18
(2) Ionic strength (L)	-1.29	2.34	-0.55	0.680	-0.64	1.17	-15.49	14.20
Ionic strength (Q)	0.55	2.74	0.20	0.873	0.28	1.37	-17.13	17.68
(3) Temperature(L)	3.46	2.34	1.48	0.378	1.73	1.17	-13.11	16.58
Temperature(Q)	3.65	2.74	1.33	0.410	1.82	1.37	-15.58	19.23
(4) Ethanol concentration (L)	-0.03	2.34	-0.01	0.991	-0.02	1.17	-14.86	14.83
Ethanol concentration (Q)	2.57	2.74	0.94	0.520	1.29	1.37	-16.12	18.69
1L by 2L	0.44	2.86	0.15	0.904	0.22	1.43	-17.96	18.40
1L by 3L	0.76	2.86	0.27	0.836	0.38	1.43	-17.80	18.56
1L by 4L	-1.19	2.86	-0.42	0.749	-0.60	1.43	-18.78	17.59
2L by 3L	-0.37	2.86	-0.13	0.918	-0.19	1.43	-18.37	17.99
2L by 4L	0.64	2.86	0.22	0.860	0.32	1.43	-17.86	18.50
3L by 4L	-0.64	2.86	-0.22	0.861	-0.32	1.43	-18.50	17.86

The analysis of variance (Table 5:15) of the permeate flux related to the second concentration step for all experiments, reveals that no factors (temperature, protein concentration, ionic strength and ETOH concentration) had a significant impact on permeate flux during this step of the UF of HSA, within the specified level for each factor. This is very similar to the results obtained for the first concentration step. The Pareto chart (Figure 5:13) shows that all factors have a p value greater than 0.05 ($p > 0.05$) and therefore all factors had no statistically significant impact on the permeate flux during this step of UF of HSA. This is further emphasized by the t value which is approximately 0; which indicated that there is little or no variation between the sample means of all the factors with respect to permeate flux. The interactive effects between factors were considered insignificant with respect to impact on permeate flux during this step. The validity of the above conclusions are supported by the linearity of the normal probability plot vs. raw residuals (data not shown). The profile for predicted values and desirability (data not shown) showed distinct trends; however, these trends are considered not significant when interpreted in relation to the p-value.

5.5 IMPACT OF SELECTED FACTORS ON PERMEATE FLUX DURING ULTRAFILTRATION

The steps of UF of HSA (first concentration step, diafiltration steps one to five and the second concentration step) and there inter-dependence on each other is important to note when analysing the results in this section. The effect of each factor (protein concentration, ETOH concentration, ionic strength and temperature) on permeate flux was evaluated over the seven steps of the UF of HSA.

5.5.1 IMPACT OF PROTEIN FEED CONCENTRATION

During this study, protein concentration is the most statistically significant factor that impacts permeate flux during UF of HSA. The effect of protein concentration on each step of the UF of HSA is discussed and exemplified according to the mechanisms of membrane and protein fouling such that an optimum protein concentration for the UF of HSA can be confirmed.

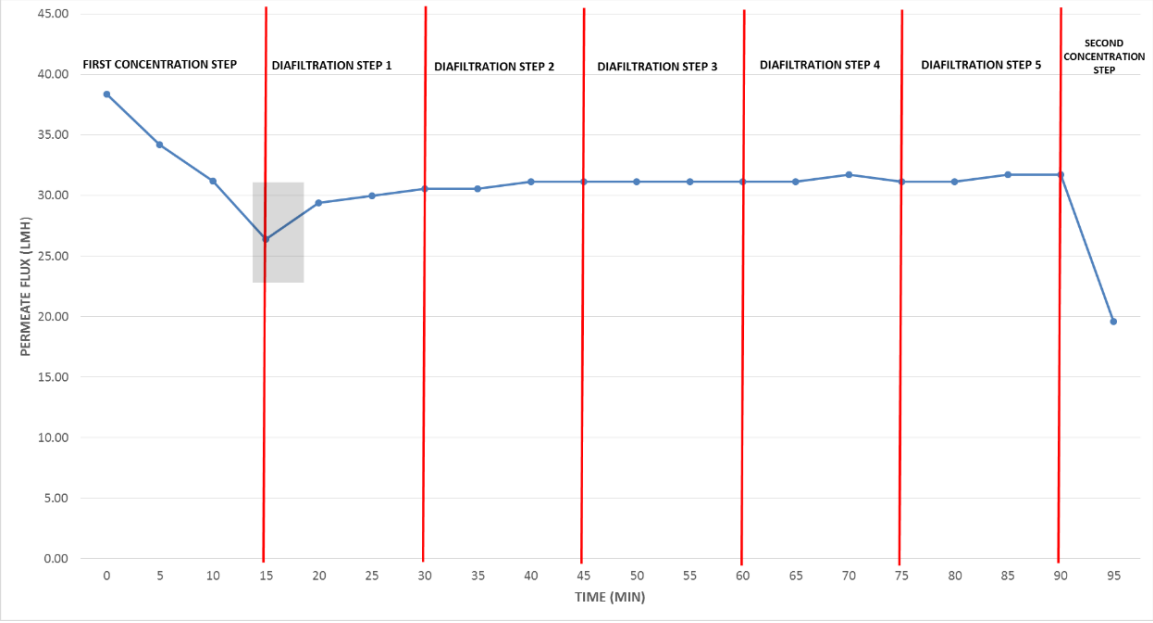


Figure 5:14: *Permeate flux (LMH) during the various steps of ultrafiltration. Note the steep decline in flux during the first concentration step; the quasi-steady state flux during diafiltration steps one to five and the decline in flux during the second concentration step.*

Protein concentration, within the range of 60g/L to 220g/L, does not have a significant impact on permeate flux during the first and second concentration steps of UF of HSA. The p-value for both steps is $p > 0.05$ (first concentration step $p = 0.0834$; second concentration step $p = 0.0874$). During the first concentration step of UF of HSA a dramatic reduction in permeate flux is noted for all experiments completed in this study. Figure 5:14, illustrates the permeate flux profile during the steps of UF for the control experiment (experiment no 26) completed during this study. Further, this figure highlights the rapid flux decline during the first concentration step. This flux decline was noted for all experiments, irrespective of start levels for each factor. This rapid flux decline during this step is attributed to the CP and MF phenomenon (Palecek and Zydney, 1994; Marshall *et al.*, 1993). During the first concentration step, the rapid flux decline is attributed to initial protein-membrane interactions whereby protein molecules are deposited onto the membrane surface thus initiating the formation of the protein monolayer or gel boundary layer. The formation of this gel boundary layer causes pore blocking or constriction which negatively impacts the hydraulic permeability of the membrane. This constriction of the pores of the membrane coupled with gel boundary layer formation and CP layer contribute to the rapid decline in flux in the first concentration step. The permeate flux effects noted in the first concentration step are exemplified by other studies including Palacek and Zydney (1994) and Marshall *et al.*, (1993).

During the second concentration step the decline in permeate flux is similar to that observed during the first concentration step. The decline in flux during this step of UF of HSA is attributed to the compaction of the gel boundary layer due to increasing protein concentration as the volume of the bulk liquid is decreased (Palecek and Zydney, 1994; Marshall *et al.*, 1993). In some experiments completed during this study, a slight increase (<2LMH) in permeate flux was noted during the transition from the first concentration step to diafiltration step one (grey zone – Figure 5:14). This increase in flux is due to an increase in TMP during the intermediate transition between these different steps.

During the diafiltration steps of UF of HSA, protein concentration is the most significant factor that influences permeate flux with p-value < 0.05 for all diafiltration steps one to five. The statistical significance of the effect of protein concentration on permeate flux, increases from diafiltration step one (p = 0.0105) to diafiltration step five (p = 0.0018). This trend of increasing statistical significance of protein concentration on permeate flux illustrates the inter-dependence of the steps of UF of HSA. Further, this trend emphasizes that the mechanisms responsible for the effects of protein concentration on permeate flux namely CP and MF dominate the entire UF process since the impact of the input variable on the measured output significantly increases from diafiltration step one to diafiltration step five. Figure 5:14 shows a quasi-steady state flux profile for the control experiment during diafiltration steps one to five. A similar trend was noted for all experiments completed during this study. During this step the gel boundary layer is completely formed and remains consistent provided that the hydrodynamic conditions and solution chemistry remain unchanged (Marshall *et al.*, 1993; Palecek and Zydney, 1994).

Table 5:16: *Table of the standardized effects for protein concentration converted in permeate flux (LMH) data and related to volumetric flow rate to indicate the overall impact on process time.*

PROTEIN CONCENTRATION (g/L)					
Permeate Flux	**DF 1**	**DF 2**	**DF 3**	**DF 4**	**DF 5**
Co-efficient (β = +/-)	-10.25	-10.13	-10.07	-10.15	-9.90
-95% (g/min)	-12.41	-11.57	-11.15	-10.87	-10.26
+95% (g/min)	-8.10	-8.69	-9.00	-9.43	-9.54
-95% (ml/min)	-12.16	-11.34	-10.93	-10.65	-10.06
+95% (ml/min)	-7.94	-8.52	-8.82	-9.24	-9.35
-95% (LMH)	-7.30	-6.80	-6.56	-6.39	6.03
+95% (LMH)	-4.76	-5.11	-5.29	-5.54	5.61
L/h (30m^2)	-218.92	-204.09	-196.73	-191.70	181.03
	-142.83	-153.37	-158.69	-166.34	168.35

The statistical analysis of the effects of protein concentration on the permeate flux during CVD (Table 5:16) indicates that the β co-efficient is negative, which shows that an increase in protein concentration results in a corresponding decrease in permeate flux within the range of the confidence interval, specified with 95% certainty. Further the magnitude of the effect of protein concentration on permeate flux (Table5:16) is considered large since the lower limit (-95%) is >4.5LMH for all diafiltration steps.

This translates to a minimum decrease in flow rate of approximately 130L/h with every 1g/L increase in protein concentration. The decrease in permeate flux as protein concentration increases is explained by examining the protein-protein interactions and the membrane-protein interactions with respect to CP and MF. Protein aggregation is generally caused by high protein concentrations in feed solutions, which causes denaturation of proteins resulting in low permeate flux during UF of proteins (Cromwell *et al.*, 2006). Also, an increase in feed concentration results in a decrease in the hydraulic permeability of the membrane, through mechanisms such as protein adsorption to membranes resulting in pore blocking and restriction. This reduction in the hydraulic permeability of the membrane and the concurrent development of the gel boundary layer results in low permeate flux during UF of proteins (Reihanian *et al.*, 1983). Protein solutions with high protein concentrations are known to exhibit gelling behaviour. The experimentally determined wall concentration of BSA for a limiting flux is approximately 300g/L; further, the wall concentration depends on that of the bulk concentration and generally increases during UF of BSA if operating conditions are maintained at a constant (Meireles *et al.*, 1991).

It was noted during this study that protein concentration had a statistically significant interactive effects with all other factors during diafiltration step five only (Appendix E, Figure E-1 to Figure E-3). The magnitude of the effects for all interactions between protein concentration and other factors was less than 1LMH (±95%). Therefore, the overall effect of the interaction on permeate flux during UF of HSA was considered small. Interestingly, β co-efficient for all interactive effects were positive. This suggests that an increase in protein concentration with any increase in the other factors will result in a corresponding increase in permeate flux. The key interpretation of this data is that protein concentration is the key factor that influences permeate flux during UF of HSA.

The fitted response surface diagrams (Appendix E, Figure E-4 to Figure E-8) for permeate flux as a function of protein concentration, for all steps during CVD indicates that the optimum permeate flux is achieved at the lowest protein concentration. These results correlate well with the desirability and predicted profiles for protein concentration, which indicates that the optimum protein concentration to achieve the highest permeate flux during UF of HSA is 60g/L; with permeate flux decreasing as

protein concentration increases within the range 60g/L to 220g/L. These results concur with the statistical data presented above, on the impact of permeate flux with protein concentration.

5.5.2 IMPACT OF ETHANOL CONCENTRATION

Ethanol is predominantly used as a precipitating agent in the manufacture of plasma proteins and must be removed from the API, by UF, prior to formulation and final filling into containers (Cohn *et al.*, 1946; Shukla and Cheryan, 2002). The UF of plasma protein solutions removes the ETOH from the bulk feed solution. During the first concentration step very little ETOH is removed from the bulk feed solution. This finding was derived from a screening experiments using similar membranes and hydrodynamic conditions as those used in the present study, the ETOH concentration (mMol/L) was determined before and after the first concentration step, for five experiments (Figure 5:15). Note that the slight variation in ETOH concentration between start (before first concentration) and end (after first concentration) of the first concentration step could be attributed to error in the experimental analysis. Further, the bulk feed solution and the filtrate samples were analysed for ETOH content after each step of the UF of HSA i.e. after first concentration step, diafiltration steps one to five and the second concentration step. The ETOH content in the bulk solution, determined after the selected steps (Figure 5:16) show clearly that ETOH is mostly removed during diafiltration steps one to five. Interestingly, the reduction in ETOH concentration represents a logarithmic decrease (R^2 > 85.00%) in concentration, with >90% of the EOTH (measured from the start of first concentration step) removed by diafiltration step three. This information is critical for proposed optimization strategies and will be discussed in following sections.

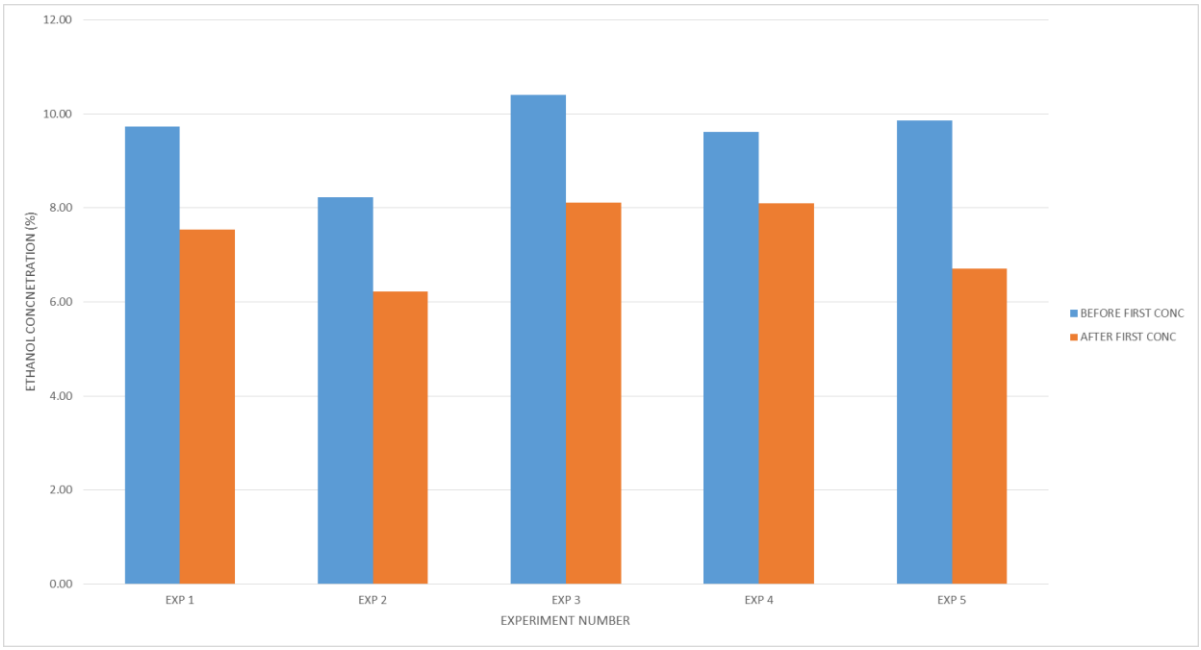


***Figure 5:15**: Graphical representation of the limited loss of ethanol (%) during the first concentration step of ultrafiltration.*

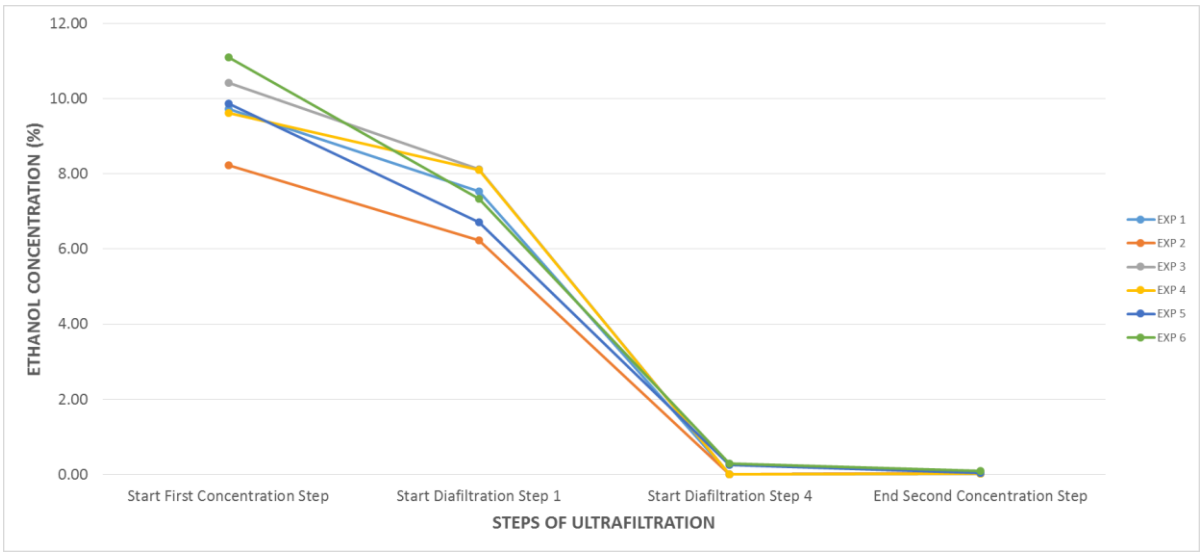


***Figure 5:16:** Graphical representation of the removal of ethanol (%) from the bulk feed during ultrafiltraiton. Note that >90% of ethanol is removed by the end of diafiltration step three.*

Ethanol concentration had a significant substantive effect on permeate flux during diafiltration steps one and two and a statistically significant effect on permeate flux during diafiltration steps three to five. The statistical significance (p-value) increases from diafiltration step one ($p = 0.0549$) to diafiltration step five ($p = 0.0173$). Figure 5:15 and Figure 5:16 shows the rate at which ETOH has been removed from the bulk feed solution; in conjunction with the statistical data it is concluded that as ETOH is removed from the bulk feed solution, the significance of the impact on permeate flux during UF of HSA increases. Diafiltration steps are inter-dependent since they occur consecutively and each cycle removes a certain concentration of unwanted macromolecules and solutes. Therefore, the effects of the selected factors (the measured outputs determined as a function of the input variables) will become more significant as the unwanted salts and solutes are removed.

The statistical analysis of the effects of ETOH concentration on the permeate flux during UF of HSA indicates that the β co-efficient is negative, which shows that an increase in ETOH concentration results in a corresponding decrease in permeate flux within the range of the confidence interval, specified with 95% certainty. During this study, the decline in permeate flux during UF of HSA with increase in ETOH concentration can be attributed to the denaturation/precipitation of proteins, resulting in increased viscosity of the feed solution. This increased viscosity of the feed solution results in the decrease of permeate flux during UF of proteins according to Darcy's Law. An analysis of the free energy of proteins in stable and denatured states shows that proteins are unstable and insoluble in polar solvents e.g. ETOH such that the degree of stability or solubility is dependent on the concentration of the solvent; were an increase in solvent concentration results in increased instability (Pace *et al.*, 2004). An increased concentration of ETOH in protein solutions causes structural and conformational changes which results in increased viscosity of the feed solution (Szymanska *et al.*, 2012). Ethanol binds water molecules more efficiently than proteins therefore high concentrations of ETOH in feed solutions cause's proteins to precipitate due to the dehydrating effect of this solvent which elicits increased electrostatic interaction between protein molecules. (Van Oss, 1989). This precipitation of proteins at high ETOH concentrations contributes to the increase in viscosity of the feed solution.

Further, the increased viscosity of the feed solution may also contribute to the higher concentration of protein molecules in the gel boundary layer due to reduced back diffusion of protein into the bulk feed solution, at constant TMP and CFV. Thus resulting in decreasing the hydraulic permeability of the UF membrane. This theory was suggested by Jaffrin and colleagues in two separate studies (Jaffrin and Charrier, 1994; Jaffrin *et al.*, 1997). The results of both studies confirmed that high ETOH concentrations induced increased viscosities of the feed solution which also caused "thickening" or increased concentration of protein molecules, of the gel boundary layer. This resulted in the decline of flux during UF of protein solutions, at fixed hydrodynamic conditions. Therefore, an increase in viscosity of the protein feed solution may have caused an increase in protein concentration of the gel layer, resulting in low permeate flux, with increasing concentration of ETOH.

Table 5:17: *Table of the standardized effects for ethanol concentration converted in permeate flux (LMH) data and related to volumetric flow rate to indicate the overall impact on process time.*

ETHANOL CONCENTRATION (%)					
Permeate Flux	**DF 1**	**DF 2**	**DF 3**	**DF 4**	**DF 5**
Co-efficient (β = +/-)	-1.96	-1.34	-1.16	-1.04	1.04
-95% (g/min)	-4.12	-2.78	-2.24	-1.76	-1.40
+95% (g/min)	0.19	0.09	-0.08	-0.32	-0.68
-95% (ml/min)	-4.04	-2.73	-2.19	-1.72	-1.37
+95% (ml/min)	0.19	0.09	-0.08	-0.31	-0.67
-95% (LMH)	-2.42	-1.64	-1.32	-1.03	0.82
+95% (LMH)	0.11	0.06	-0.05	-0.19	0.40
L/h (30m^2)	-72.66	-49.06	-39.47	-31.01	24.70
	3.43	1.67	-1.43	-5.65	12.02

Table 5:17 indicates the effects of EOTH concentration on permeate flux per diafiltration step; the flux values are translated into flow rates (L/h) to indicate the overall impact on process time. The +95% confidence limit for diafiltration step one is -2.42LMH; for all other diafiltration steps this limit is < -2.00LMH. In diafiltration steps one to three, the magnitude of the effect of ETOH concentration is very limited possibly because the range of ETOH concentration selected for this study was very narrow (0% to 10% - v/v). This, coupled with the reducing concentration of ETOH from diafiltration step one to five will account for the low magnitude of the effect of

ETOH concentration. In diafiltration step four and five, the +95% confidence limit is approximately 1.00LMH. The magnitude of the effects of ETOH concentration on permeate flux, although considered significant, are very small. This reduced effect of ETOH concentration in diafiltration steps four and five is expected since >90% of ETOH is removed before diafiltration step four (Figure 5:16). In all experiments cited above, including Jaffrin and Charrier (1994) and Jaffrin *et al.*, (1997), the limiting effects of ETOH concentration on permeate flux was conducted using ETOH concentrations at a minimum of 20% (v/v) since the authors recognized that the viscosity of albumin-ETOH solutions at 20°C showed minimal increase in viscosity (<2.00mPa.s) with increase in ETOH concentration (up to 30% v/v); across a range of protein concentrations (0g/L to 20g/L) with fixed hydrodynamic conditions (Table 3:3). Further, Szymanska *et al.*, (2012) showed that from 0% to 60% (v/v) ETOH concentration, the rate of viscosity increase is slower than compared to viscosity increase with ETOH concentration >60%. The range of this study was 0% (v/v) to 10% (v/v) and therefore, the results of this study is showing a minimal, limiting effect on permeate flux during UF of HSA due to the narrow range of the factor.

The fitted response surface diagrams (Appendix E, Figure E-9 to Figure E-13) for permeate flux as a function of ETOH concentration and protein concentration, for all steps of constant volume diafiltration indicates that the optimum permeate flux is achieved at low ETOH concentrations. These results correlate well with the desirability and predicted profiles for ETOH concentration, which indicates that the optimum ETOH concentration to achieve the highest permeate flux during UF of HSA lies in the range 0% (v/v) to 7.75% (v/v); with permeate flux decreasing as ETOH concentration increases. However, the magnitude of the effect on permeate flux within the range of the factor is small.

The optimization of ETOH concentration with respect to maximizing permeate flux must consider the following:

- Mother liquor content of dissolve Fraction V paste is approximately 5-7% (v/v). Addition of ETOH (96%, v/v) during dissolution to increase concentration to approximately 10% (v/v)

- During this dissolution phase, the product temperature is reduced to -3.5°C ± 1°C
- Removal of 10% WFI-ETOH admixture for dissolution of HSA has a regulatory and process impact on upstream process of depth filtration. Therefore a change will require process optimization, validation and regulatory approval.
- Limited cost saving with respect to decreasing ETOH concentration.

5.5.3 IMPACT OF TEMPERATURE

Temperature is a critical process parameter in UF of proteins since it impacts the physic-chemical properties of both the membrane and the protein of interest (Mo *et al.*, 2008). During this study, temperature was varied within the range 5°C to 25°C, and the impact on permeate flux, across the range of several other parameters, was statistically determined.

Table 5:18: *Table of the standardized effects for temperature converted to permeate flux (LMH) data and related to volumetric flow rate to indicate the overall impact on process time.*

TEMPERATURE (°C)					
Permeate Flux	**DF 1**	**DF 2**	**DF 3**	**DF 4**	**DF 5**
Co-efficient (β = +/-)	2.26	2.18	2.06	2.05	1.90
-95% (g/min)	0.10	0.74	0.98	1.33	1.54
+95% (g/min)	4.42	3.62	3.14	2.77	2.26
-95% (ml/min)	0.10	0.73	0.96	1.30	1.51
+95% (ml/min)	4.33	3.55	3.07	2.71	2.21
-95% (LMH)	0.06	0.44	0.58	0.78	0.90
+95% (LMH)	2.60	2.13	1.84	1.63	1.33
L/h (30m^2)	1.80	13.13	17.30	23.42	27.10
	77.89	63.85	55.35	48.78	39.79

Temperature had a statistically significant effect on permeate flux during all diafiltration steps. The statistical significance (p-value) increases from diafiltration step one (p = 0.0477) to diafiltration step five (p = 0.0095). This increase in statistical significance across CVD is similar to that noted for ETOH concentration on permeate flux. Therefore, the same inference can be concluded i.e. the interdependence of the consecutive diafiltration steps increases the significance of the effects of the factor on the measured outputs, during the steps of diafiltration.

The statistical analysis of the effects of temperature on the permeate flux during UF of HSA (Table 5:18) indicates that the β co-efficient is positive, which shows that an increase in temperature results in a corresponding increase in permeate flux within the range of the confidence interval, specified with 95% certainty. The positive impact of temperature on permeate flux can be attributed to the low viscosity of HSA solutions at high temperature. The interpretation of Darcy's law suggests that the less viscous a solution, the greater the flow through the membrane (Jonsson and Tragardh, 1990). Further, the Arrhenius Equation confirms the relationship between viscosity and temperature by showing that as temperature increases, viscosity decreases (Monkos, 1996). The reduced viscosity of solutions at high temperatures increases the back diffusion of solutes away from the membrane i.e. increases the diffusion co-efficient of solutes, causing a reduction in the concentration of solutes at the gel boundary layer, resulting in increased permeate flux during UF of proteins (Mo *et al.,* 2008). The Monkos study of 1996 showed the logarithmic reduction in viscosity of a BSA solution of concentration 335g/L as temperature increases, up to a maximum temperature of 45°C. Masuelli (2013) showed a similar phenomenon with BSA, the viscosity of BSA solutions at two concentrations (20.0g/L; 367.1g/L) showed the same effect i.e. viscosity of the solution decreased as temperature increased. Further, this study elucidated the correlation between protein concentration and temperature on viscosity in that the higher protein concentration solution showed a higher viscosity; which decreased as temperature was increased (Masuelli, 2013). The effect of operating temperature on solution viscosity has been conclusively proven for other solutes e.g. cherry juice (Wang *et al.,* 2005)

The impact of ETOH on the viscosity of HSA has been explained in section 5.2.2. Literature supports the hypothesis that an increase in ETOH concentration causes an increase in the viscosity of HSA solutions, which results in decrease in permeate flux during UF of HSA (Syzmanska *et al.,* 2012; Jaffrin and Charrier, 1994; Jaffrin *et al.,* 1997). The removal of ETOH in the bulk feed solution, as a consequence of constant volume diafiltration, will result in a limited decrease of the viscosity of the feed solution. Therefore, as ETOH is removed from the bulk feed, the impact of temperature on the steps of UF of HSA becomes more significant. Thus the statistical significance

of temperature on permeate flux increases from diafiltration step one to diafiltration step five.

The magnitude of the effects of temperature on permeate flux, within the range 5°C to 25°C, during UF of HSA is less than +3.00LMH, for all diafiltration steps, at the +95% confidence interval. This translates to an overall impact on flow rate of less than 100L/h, for an UF membrane surface area of $30m^2$. Therefore, although the impact of temperature on permeate flux during UF of HSA is considered significant, the magnitude of the impact is considered small, with limited impact on permeate flux during UF. The temperature range (5°C to 25°C) selected for this study, could be too narrow, therefore the effect of temperature on permeate flux is limited. HSA solubility within this temperature range is considered high and stable, therefore, MF due to protein denaturation is limited and thus the impact of temperature is considered statistically significant, with minimal impact but with increase in permeate flux as temperature increases. The theoretical basis for HSA stability within this temperature, as a function of reversible/irreversible protein unfolding, will be explained further in paragraphs below.

The fitted response surface diagrams (Appendix E) for permeate flux as a function of temperature and protein concentration for all steps of CVD indicate that the optimum permeate flux is achieved between 20°C to 25°C, for all protein concentrations within the range 60g/L to 220g/L. The response surface diagrams correlates well with the predicted and desired value for temperature which indicates that the optimum temperature to achieve the highest flux during UF of HSA is 25°C. The predicted and desired value analysis also showed that a temperature set point of 20°C, with a desirability value close to 1.000, may also be considered in the optimization analysis for the UF of HSA. It is a well-known fact that proteins are stable at low temperatures and therefore, the lower temperature set point might be favourable. However, the conformational and structural analysis of HSA suggests that this protein only undergoes irreversible change at temperatures greater than 45°C, with reversible change occurring at temperatures between lower than 45°C. The increase in temperature is known to cause the conformational and structural change of HSA from the stable α helical structure to β sheets, characterized by a general unfolding of the protein towards the denatured state. HSA is known to be stable in temperatures less than 30°C. The results

obtained in this study are similar to those obtained by Campbell *et al.*, (1993) who achieved a similar temperature set point for the UF of α amylase.

5.5.4 IMPACT OF pH AND IONIC STRENGTH

During this study, pH was maintained at pH 7.2 ± 0.2 for all experiments completed. This physiological pH maintains optimum stability of the HSA in the native state. Also, the pH 7.2 ± 0.2 is further away from the IEP of HSA, determined to be pH 4.7. This ensures that the impact of low permeate flux at IEP during UF of HSA is negligible for experiments completed during this study. Flux during UF of proteins is greatly reduced at the IEP of the protein of interest (Swaminathan *et al.*, 1981; Fane *et al.*, 1983; Salgin *et al.*, 2006). This reduction in flux at the IEP is explained by decreased protein-protein electrostatic repulsion. Protein adsorption to the membrane is a maximum at the IEP since HSA has a net zero charge thus increasing MF through enhanced protein adsorption onto the PES membrane, which is negatively charged at pH 4.7 (Wang and Tang, 2011; Lim and Mohammad, 2010). When the pH of a solution deviates towards lower pH the protein molecules become protonated (Equation 5:1a, b) and carry a net positive charge whilst the PES membrane has a weak negative charge. This results in an increase in protein adsorption to PES membranes. Therefore, completing UF of HSA at low pH is not feasible due to the increased protein fouling and corresponding decrease in permeate flux. In a key study, Salgin *et al.*, (2006) showed that adsorption of protein to 10kDa PES membranes are even greater at pH<IEP. When pH of a solution deviates from the IEP of a protein towards higher pH, the membrane and the protein both have negative surface charge due to the dissociation of the carboxyl group of the protein (Equation 5:2a, b). This increases the force of electrostatic repulsion between proteins and the membrane surface (true only for negatively charges membranes such as those used in this study); MF is subsequently reduced by the reduction of protein adsorption to the membrane surface (Lim and Mohammad, 2010). When the pH of a solution is lower than the IEP of the protein, the protein is protonated (Equation 5:1a, b) and thus positively charged. Therefore, if negatively charged membranes are used with positively charged proteins, MF will increase due to the electrostatic attraction between the positively charged proteins and the negatively charged membrane (Lim and Mohammad, 2010).

$$R - NH^3 + OH^- \rightarrow R - NH^2 + H_2O \quad \text{a}$$

$$R - COOH + OH^- \rightarrow R - COO^- + H_2O \quad \text{b}$$

Equation 5:1a,b: *Chemical equations showing the dissociation of the carboxyl group of proteins in solutions at pH>IEP, resulting in net negative charge of the proteins (Lim and Mohammad, 2010).*

$$R - NH^2 + H^+ \rightarrow R - NH_3^+ \quad \text{a}$$

$$R - COO^- + H^+ \rightarrow R - COOH \quad \text{b}$$

Equation 5:2a,b: *Chemical equations showing the protonation of proteins in solutions at pH<IEP, resulting in net positive charge of the proteins (Lim and Mohammad, 2010).*

The UF of protein solutions removes various ions from the bulk feed solution including sodium, potassium, citrate and aluminium. Similar to ETOH, little or no reduction in ion concentration is noted during the first concentration step. In the studies conducted by this laboratory described in the section above, the ion concentration of four key ionic species (potassium; sodium; citrate; aluminium) was determined before and after the first concentration step, for five experiments. The concentration of potassium (Figure 5:17) and sodium (Figure 5:18) decreased not more than 2mol/L during the first concentration step. A similar trend was noted for citrate and aluminium (data not shown). Note that the slight variations in measured ion concentration before and after the first concentration step could also be due to experimental error. The analysis of the removal of ions during UF of HSA showed that >90% of measured ions was removed from the bulk feed solution during the CVD step (Figure 5:19 and Figure 5:20). The majority of these ions were removed by diafiltration step three. This information is critical for proposed optimization strategies and will be discussed in following sections.

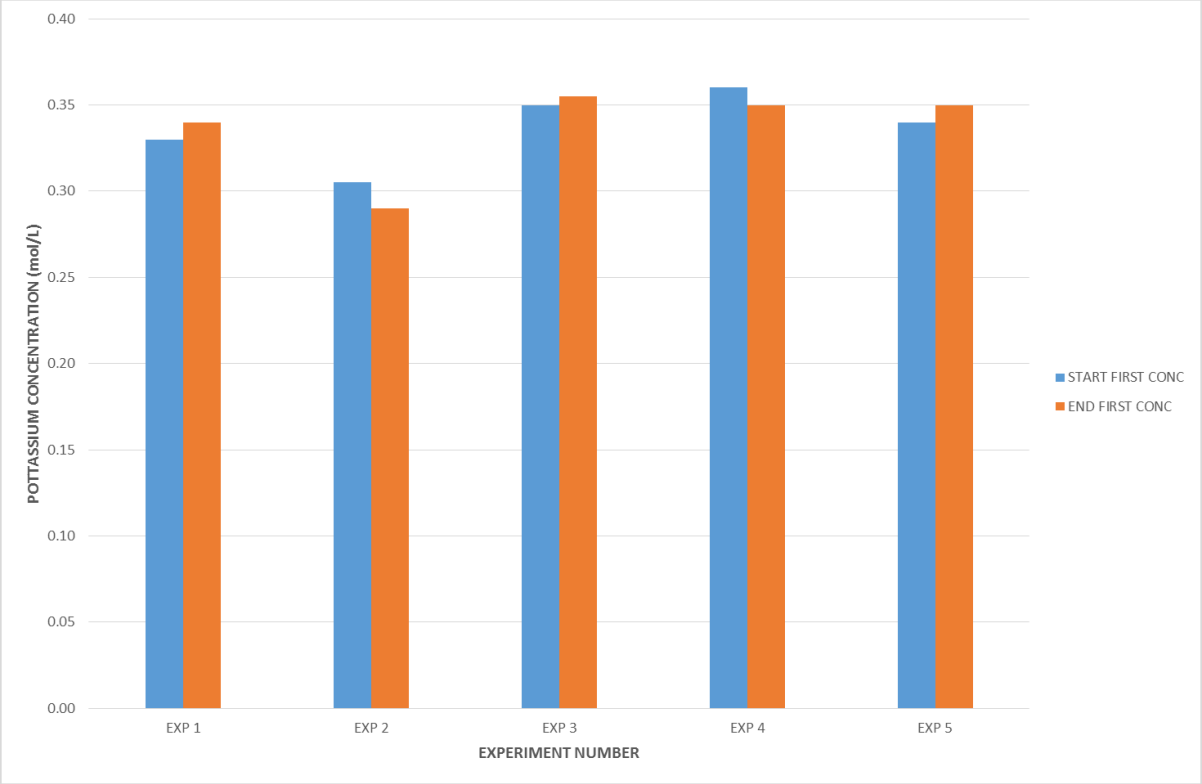


Figure 5:17: *Graphical representation of the minimal loss of potassium (mol/L) during the first concentration step.*

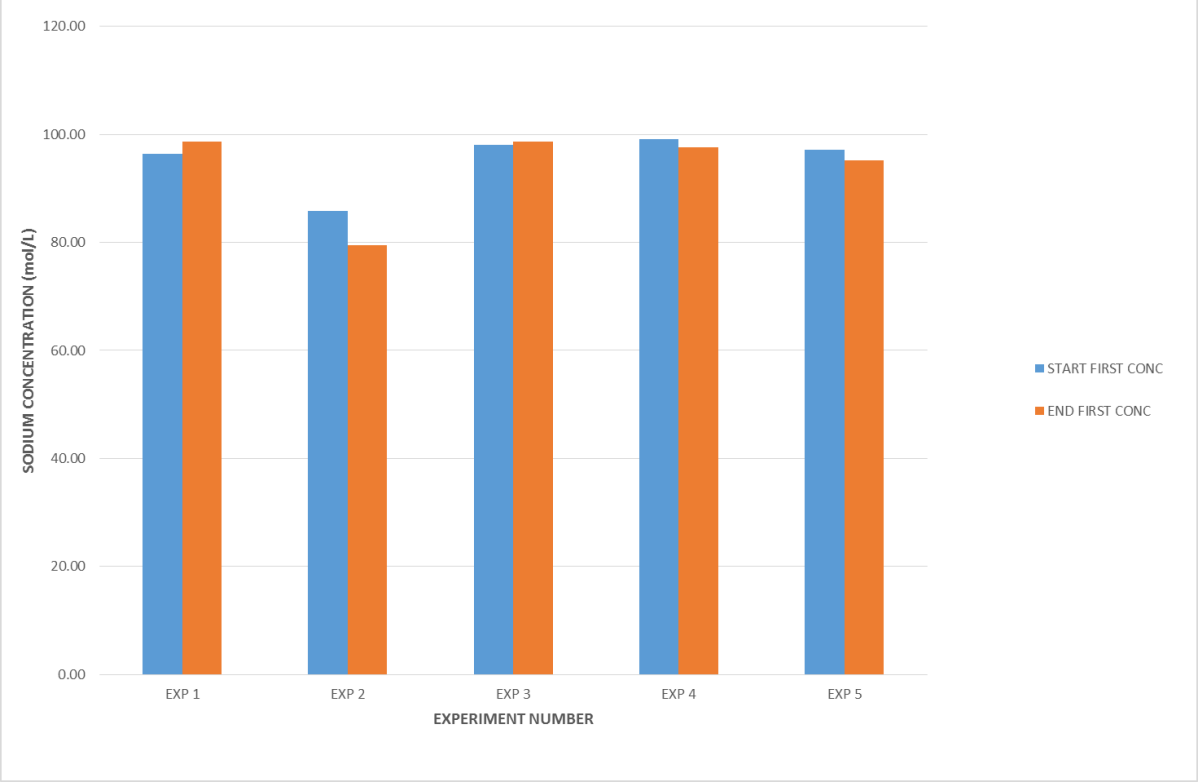


Figure 5:18: *Graphical representation of the minimal loss of sodium (mol/L) during the first concentration step.*

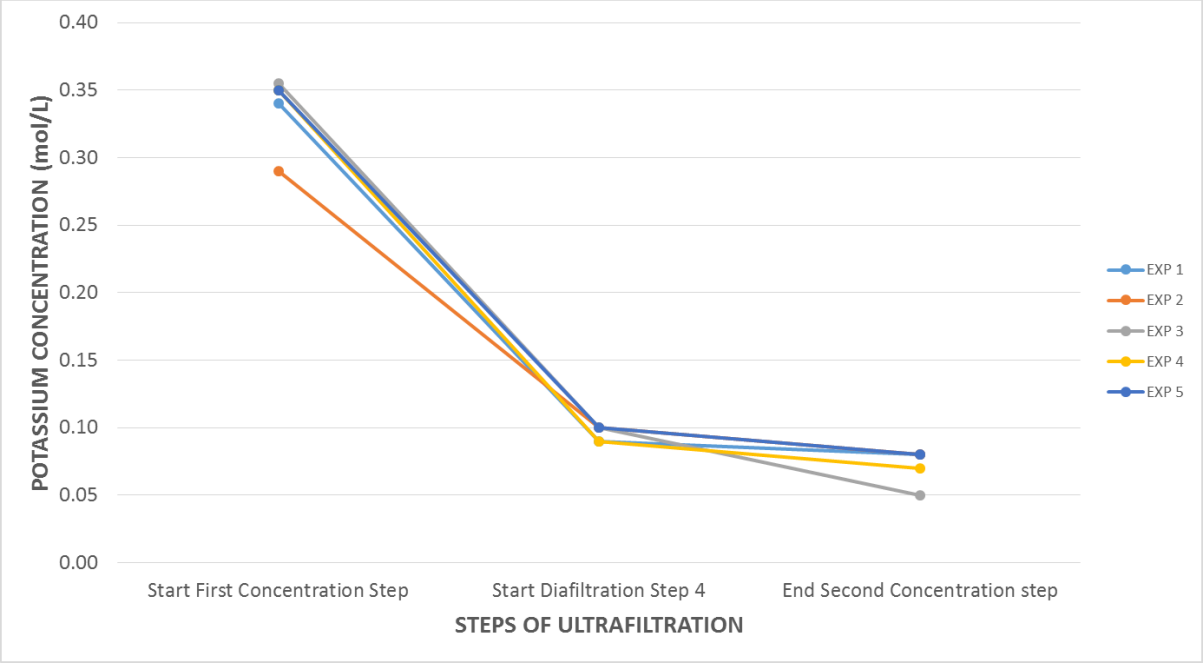


Figure 5:19: *Graphical representation of the removal of potassium (mol/L) from the bulk feed solution during ultrafiltration. Note that >90% of the potassium is removed by the end of diafiltration step three.*

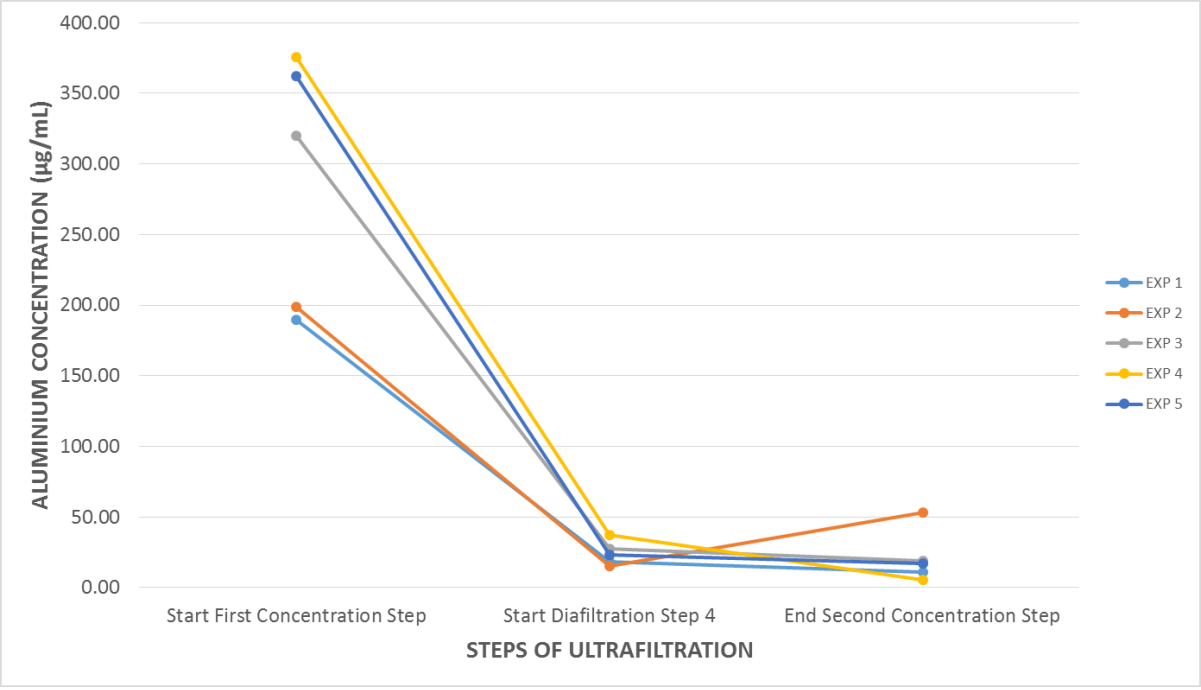


Figure 5:20: *Graphical representation of the removal of aluminium (mol/L) from the bulk feed solution during ultrafiltration. Note that >90% of the aluminium is removed by the end of diafiltration step three.*

During this study, NaCl diluent of varying ionic strengths (0M – 3M) was used to determine the impact of ionic solutions on permeate flux during the UF of HSA. Ionic strength had a significant substantive effect on permeate flux during diafiltration step four and a statistically significant effect on permeate flux during diafiltration step five. During diafiltration steps one to three, ionic strength did not have a statistically significant impact on permeate flux. High ionic strength solutions, such as those used in this study, exhibit an electrostatic shielding effect of the protein molecules which decreases the protein-protein electrostatic repulsion force and the protein-membrane electrostatic attraction force (Salgin, 2007). Therefore the statistically significant impact of ionic strength is only noted in diafiltration step five since by this step in UF of HSA the polar solvent (ETOH) and other competing ions e.g. aluminium and citrate are reduced to low concentrations and there is less competition for binding of NaCl ions to the HSA protein molecule. The electrostatic shielding effect, caused by the binding of NaCl ions (high concentration greater than physiological salt concentrations) to the protein molecule, induces the decrease in permeate flux during UF of HSA (Zhang and Cremer, 2006).

The statistical analysis of the effects of ionic strength on the permeate flux during UF of HSA indicates that the β co-efficient is negative, which shows that an increase in ionic strength results in a corresponding decrease in permeate flux within the range of the confidence interval, specified with 95% certainty. Several authors have clearly demonstrated the reduction in permeate flux during UF of protein solutions with high ionic strength solutions where pH of the protein solution was above the isoelectric point pH. Increase of ionic strength by the addition of NaCl solutions during constant volume diafiltration results in a charge screening or masking effect (electrostatic shielding) of the ions that reduces the overall net charge of the protein molecule which causes them to act as uncharged molecules. Therefore, this reduces the electrostatic repulsion with respect to protein-protein and protein-membrane interactions, by double layer compression, resulting in increasing protein adsorption to the membrane and protein fouling which has a net effect of reducing permeate flux during UF of proteins (Lim and Mohammad, 2010; Wang and Tang, 2011). Palecek *et al.*, (1993) provided conclusive evidence that increase in ionic strength above and below the IEP of BSA decreases the permeability of the gel layer thus decreasing permeate flux. The model explaining these interactions were similar to those expresses by Lim and Mohammad

(2010). The analysis of the dominant forces that influence the movement of a protein in a solution during UF towards the gel boundary layer shows that the repulsive force between proteins is a function of the hydrodynamic radius of the protein as well as the Debye length; Debye length is inversely proportional to the square root of the solution ionic strength (Palecek and Zydney, 1994; Palecek *et al.*, 1993).Permeate flux is increased when electrostatic repulsion between protein molecules is increased since the increased diffusive back transport of protein molecules to the bulk liquid decreases the concentration of the protein molecules at the membrane interface (Peeva *et al.*, 2012).

Table 5:19: *Table of the standardized effects for ionic strength converted in permeate flux (LMH) data and related to volumetric flow rate to indicate the overall impact on process time.*

IONIC STRENGTH (M)		
Permeate Flux	**DF 4**	**DF 5**
Co-efficient (β = +/-)	-0.665	-0.85
-95% (g/min)	-1.384	-1.21
+95% (g/min)	0.054	-0.49
-95% (ml/min)	-1.36	-1.18
+95% (ml/min)	0.05	-0.48
-95% (LMH)	-0.81	0.71
+95% (LMH)	0.03	0.29
L/h (30m^2)	-24.41	21.27
	0.95	8.58

Table 5:19 indicates the effects of ionic strength on permeate flux per diafiltration step; the flux values are translated into flow rates (L/h) to indicate the overall impact on process time. The +95% confidence limit for diafiltration steps four and five is less than 1LMH. The magnitude of the effects of ionic strength on permeate flux, although considered significant, are very small.

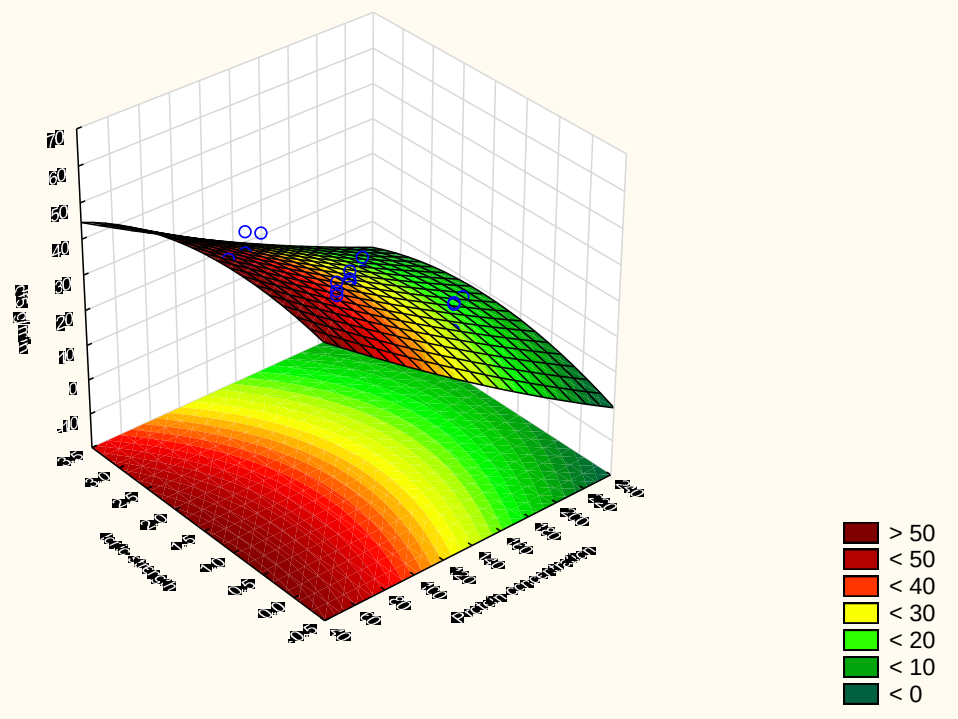


Figure 5:21: *Fitted surface response diagram for diafiltration step five showing protein concentration as a function of ionic strength and flow rate.*

The fitted response surface diagrams (Figure 5:21) for permeate flux as a function of ionic strength and protein concentration for diafiltration steps four and five of constant volume diafiltration indicate that the optimum permeate flux is achieved with 0M to 1.5M NaCl, for all protein concentrations within the range 60g/L to 220g/L. The response surface diagrams correlates well with the predicted and desired value for temperature which indicates that the optimum ionic strength to achieve the highest flux during UF of HSA lies within the stated range. For the optimization of the UF of HSA, diafiltration solutions with 0M ionic strength will be used. The results of this study showed that the range of ionic strength selected for this study was too broad since high ionic strength solutions have a screening effect on protein molecules.

5.6 OPTIMIZATION OF THE ULTRAFILTRATION OF HSA

The primary goal of optimization and innovation strategies relating to the UF of HSA include reducing operating costs and energy consumption by driving the process towards optimal operational control (Paulen *et al.*, 2015). The key elements relating to the optimal operational control of the UF of HSA are classified into two categories. In the

first category the key factors (TMP, protein concentration, ETOH concentration, ionic strength, pH, temperature and stirring rate) that influence UF of HSA are optimized to establish the critical conditions to complete the process. In the second category, diluent minimization strategies are considered and this involves maximizing the overall productivity of the UF of HSA where productivity is defined as the kilograms of protein processed per hour. Productivity is not influenced by UF performance (permeate flux) but also by other routine operations including time taken to clean membranes after use, availability of start raw materials (Fraction V paste) for UF and availability of human resources to complete tasks e.g. day shift and night shift teams.

5.6.1 OPTIMIZATION OF FEED CONCENTRATION

Protein concentration is the key factor that influences permeate flux during UF of HSA. Therefore, the optimal set point of this factor is critical to achieve the purpose of this optimization strategy. The statistical analysis of the data collected during this study suggests that the optimum protein concentration for maximum permeate flux is 60g/L. However, in optimization strategies for UF of HSA, the optimum protein concentration of the bulk feed solution is dependent on the trade-off between flux and diafiltration diluent (Millipore Application Note, 2013). It has been established that CP and MF are the key factors that cause the decline of permeate flux during UF of HSA. Therefore, one can use the gel polarization model (Equation 3:1) to determine the maximum protein concentration at which to complete UF of HSA (Asbi and Cheryan, 1992). The gel concentration (C_g) of the protein solution is defined as the value of the bulk concentration (C_b) when permeate flux is zero (Asbi and Cheryan, 1992; Millipore Application Note, 2013). Once C_g is determined, the optimum protein concentration (C_{opt}) at which ultrafiltration can occur is C_g divided by the Napier's number $\varepsilon = 2.718$ (Asbi and Cheryan, 1992; Millipore Application Note, 2013).

$$C_{OPT} = \frac{C_G}{2.718}$$

C_{opt} = optimum protein concentration at which to complete UF

C_g = gel concentration

Equation 5:3: *Determination of the optimum protein concentration to complete ultrafiltration at optimum permeate flux with minimal membrane fouling. Cg is the gel concentration and 2.718 is Napier's Number.*

Equation 5:3 can only be used under the following conditions:

- When proteins are 100% rejected
- Plot of Flux (LMH) vs. protein concentration has a regression co-efficient of greater than 90.00% such that the concentration at zero flux can be accurately determined

The above conditions have been satisfied for this study.

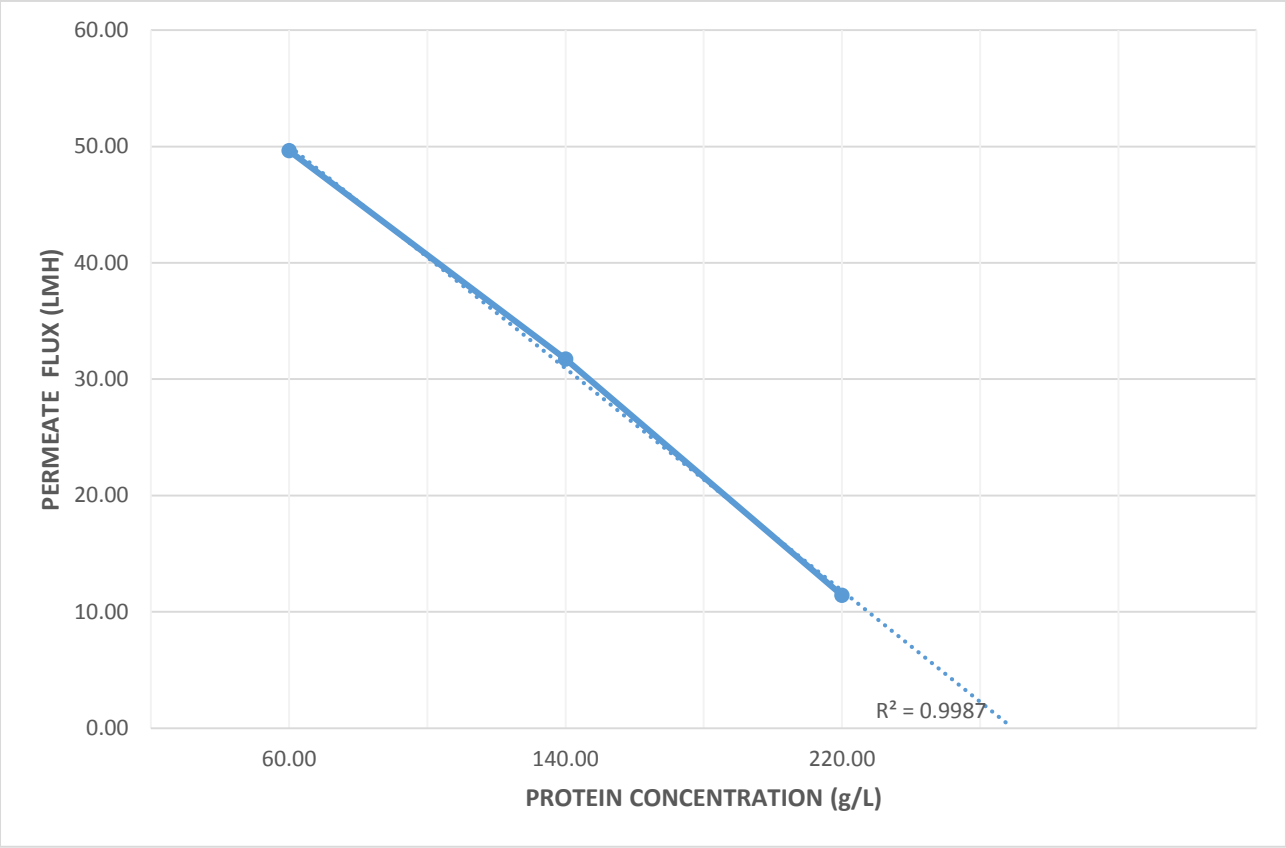


Figure 5:22: *Extrapolation of C_g from a plot of permeate flux (LMH) vs. protein concentration (g/L); according to the gel concentration model.*

The plot of Flux (LMH) vs. concentration for three experiments (experiment numbers 17, 25 and 18) completed in this study is shown in Figure 5:22. Note that the experiments 17, 25 and 18 where selected to determine the gel concentration of the ultrafiltration of HSA since the levels of all factors were identical except protein concentration, which was varied at 60g/L for experiment 17; 140g/L for experiment 25 and 220g/L for experiment 18. A plot of permeate flux (LMH) vs. protein concentration (g/L) using data from this study revealed a R^2 value approaching 100%; extrapolating permeate flux to zero, C_g = 270g/L. This value of 270g/L correlates well with the C_g value established by Jaffrin and Charrier (1992) of 300g/L. Using Equation 5:3, the optimum protein concentration to complete UF of HSA is 99.34g/L. Considering error in process equipment and related volumetric and mass measurement devices, the range for optimum protein concentration to complete UF of HSA is 100g/L ± 10g/L. The deviation of ± 10g/L is equivalent to a variation of 10% from the expected value and is congruent with current process specifications, which are validated and GMP compliant.

It is important to note that according to the process steps described in Figure 2:4, the step following the dissolution of Fraction V paste is depth filtration; this unit operation must be completed at a protein concentration of 60g/L and has not been optimized as part of this study. By using a fixed batch start volume of 1150L (which is based on the maximum capacity of the current tank used for this process), the limiting protein concentration defined for the depth filtration unit operation and the yield of HSA from Fraction V paste, the theoretically determined mass of Fraction V paste to be dissolved in the WFI-ETOH admixture is 276kg. This represents an increase on current batch size (190kg of Fraction V paste) of 31.16%. Considering the current fill volumes per vial and the volume of HSA solution after formulation, the batch size increase with respect to number of vials filled is approximately 6%.

5.6.2 OPTIMIZATION OF ETHANOL CONCENTRATION

The optimum ETOH concentration that provided the highest permeate flux was within the range 0% to 7.75% (v/v). The magnitude of the effect is considered small (<2LMH). During the dissolution of Fraction V paste in WFI, ETOH (96% v/v) is added to a final concentration of 10% (v/v); note that the dissolved Fraction V paste has a mother liquor ETOH content of between 5-7% (v/v). Therefore, a limited amount

(< 45L; dependent on mass of paste dissolved) of 96% ETOH is added to the dissolved Fraction V paste solution to achieve the final concentration of 10% (v/v). The addition of ETOH to final concentration of 10% (v/v) is important since it lowers the freezing point of the solution whilst preventing freezing of the protein solution during the next unit operation which is depth filtration. This depth filtration process is carried out at a temperature of -3.5°C ± 0.2°C. This study provides evidence for the reduction of ETOH concentration to within the range 0% to 7.75% (v/v) which is within the range of the mother liquor ETOH content of dissolved Fraction V paste. This suggests that no 96% (v/v) ETOH will be required to increase ETOH concentration to 10% (v/v). This process change will impact the upstream unit operation of depth filtration, which will require the validation and regulatory approval of a process change in temperature from the current set point of -3.5°C ± 0.2°C since the freezing point of ETOH mixtures of 5-7% (v/v) is <-3.5°C. Considering the limited economic benefit, coupled with the limited benefit in increasing permeate flux by reducing ETOH content to within the range of 0% to 7.75% (v/v) and the process development, optimization and validation efforts required to adjust downstream unit operations; it is recommended that the EOTH concentration for the UF of HSA remain at 10% (v/v).

5.6.3 OPTIMIZATION OF TEMPERATURE

The optimum temperature for the UF of HSA as statistically determined during this study is 25°C. Also, the temperature set point of 20°C was also considered to be favourable. Currently, the UF of HSA is completed at 20°C ± 2°C. The optimum statistically determined temperature set point and the current process temperature set point are within range and therefore the temperature set point for this process will not be changed.

5.6.4 OPTIMIZATION OF pH AND IONIC STRENGTH

The optimum pH for the UF of HSA is pH 7.2 for reasons established in Section 3.4.1. The statistical analysis of the data compiled during this study revealed that optimum ionic strength to achieve maximum permeate flux lies within the range 0M to 1.5M for NaCl solutions. However, the magnitude of the effect on permeate flux was considered small (<1LMH). Low Na content PDMP's have significant physiological benefit, therefore, the maximum allowed concentration of Na in HSA produced by NBI is

100mMol. Since the overall benefit of introducing a NaCl diafiltration diluent will result in little increase in permeate flux whilst increasing the Na content in the final product solution, it is recommended that the current process continue to use WFI as a diafiltration diluent. The use of a NaCl buffer will increase process cost, with no benefit of reducing process time by increasing permeate flux during UF of HSA.

5.6.5 OPTIMIZATION OF PRODUCTIVITY

The productivity of the UF of HSA is limited by the availability of the starting raw material, which is Fraction V paste. The maximum mass of Fraction V paste produced per week is approximately 520kg. In the evaluation of an optimized strategy for UF of HSA the following assumptions are considered to eliminate the inconsistencies that might occur in a dynamic manufacturing environment. These assumptions are:

- Operator scheduling constraints have been removed and the UF of HSA is deemed a 24 hour operation encompassing two shifts.
- Assume that raw materials are readily available. Note that this is important since Fraction V paste (starting raw material for HSA UF) is produced through fractionation from Fraction IV/V by the Kistler and Nitschmann (1962) method and although large quantities are readily available a 24 hour, two shift operation will steadily deplete inventory and therefore production of Fraction V paste must satisfy at minimum the rate at which it is used.
- Pre-UF of HSA activities e.g. storage buffer removal through rinsing with WFI and NWP measurements takes approximately 120 minutes to complete; the time for these activities have been factored into the overall time schedule.
- Post-UF of HSA activities e.g. CIP of membranes take approximately 90 minutes to complete; the time for these activities have been factored into the overall time schedule. The overall process time calculated above was used to determine how many batches can be completed in 24 hours; transposed to a five day working week transposed to a working year. This value (total batches produced in a year) was used to determine amount of protein mass produced in one year.
- Total process time was determined for a standard batch size of 1150L volume, which is based on the capacity of the current tank used for this process.

- Permeate flux was determined using the mass flow rates documented in this study and a nominal $10m^2$ membrane surface area, which is the maximum surface area of the current UF cassette holder.

Table 5:20: *Optimization of productivity with respect to total number of batches produced per year, translated to kg protein processed per year.*

StandardRun	Protein concentration (g/L)	Average df g/min	Total process time (hours)	Theoretical Number of runs/24 hr shift	Practical Number of runs/24 hr shift	Mass of protein (kg) per batch	Batches per week (optimum)	Batches per year (optimum)	Mass of protein (kg) per year (optimum)	Mass of protein (kg) per week (optimum)	Practical Batches per week (current)	Batches per year (current)	Mass of protein (kg) per year (current)	Mass of protein (kg) per week (current)
17	60.00	49.62	5.92	4.06	4.00	72	20	1040	74880	1440.00	7	364	26208	504
2	100.00	36.71	6.77	3.55	3.00	120	15	780	93600	1800.00	4	208	24960	480
7	100.00	44.49	6.20	3.87	3.00	120	15	780	93600	1800.00	4	208	24960	480
6	100.00	35.01	6.93	3.46	3.00	120	15	780	93600	1800.00	4	208	24960	480
8	100.00	38.61	6.61	3.63	3.00	120	15	780	93600	1800.00	4	208	24960	480
4	100.00	41.28	6.41	3.75	3.00	120	15	780	93600	1800.00	4	208	24960	480
1	100.00	44.70	6.18	3.88	3.00	120	15	780	93600	1800.00	4	208	24960	480
5	100.00	34.79	6.95	3.45	3.00	120	15	780	93600	1800.00	4	208	24960	480
3	100.00	42.90	6.30	3.81	3.00	120	15	780	93600	1800.00	4	208	24960	480
23	140.00	33.19	7.12	3.37	3.00	168	15	780	131040	2520.00	3	156	26208	504
22	140.00	34.45	6.98	3.44	3.00	168	15	780	131040	2520.00	3	156	26208	504
26 (C)	140.00	29.24	7.60	3.16	3.00	168	15	780	131040	2520.00	3	156	26208	504
19	140.00	24.84	8.33	2.88	2.00	168	10	520	87360	1680.00	3	156	26208	504
25 (C)	140.00	31.73	7.28	3.30	3.00	168	15	780	131040	2520.00	3	156	26208	504
20	140.00	28.93	7.65	3.14	3.00	168	15	780	131040	2520.00	3	156	26208	504
21	140.00	27.88	7.80	3.08	3.00	168	15	780	131040	2520.00	3	156	26208	504
24	140.00	28.10	7.77	3.09	3.00	168	15	780	131040	2520.00	3	156	26208	504
11	180.00	26.78	7.98	3.01	3.00	216	15	780	168480	3240.00	2	104	22464	432
10	180.00	17.51	10.35	2.32	2.00	216	10	520	112320	2160.00	2	104	22464	432
15	180.00	23.63	8.58	2.80	2.00	216	10	520	112320	2160.00	2	104	22464	432
13	180.00	19.51	9.65	2.49	2.00	216	10	520	112320	2160.00	2	104	22464	432
14	180.00	17.82	10.24	2.34	2.00	216	10	520	112320	2160.00	2	104	22464	432
12	180.00	27.04	7.94	3.02	3.00	216	15	780	168480	3240.00	2	104	22464	432
9	180.00	18.25	10.08	2.38	2.00	216	10	520	112320	2160.00	2	104	22464	432
16	180.00	18.80	9.88	2.43	2.00	216	10	520	112320	2160.00	2	104	22464	432
18	220.00	11.42	14.01	1.71	1.00	264	5	260	68640	1320.00	2	104	27456	528

The information described above was used to determine a productivity table for each experiment conducted based on the average permeate flux during diafiltration for that experiment. The total process time and therefore the kilograms of protein produced per year was calculated and statistically analysed. The results are discussed below.

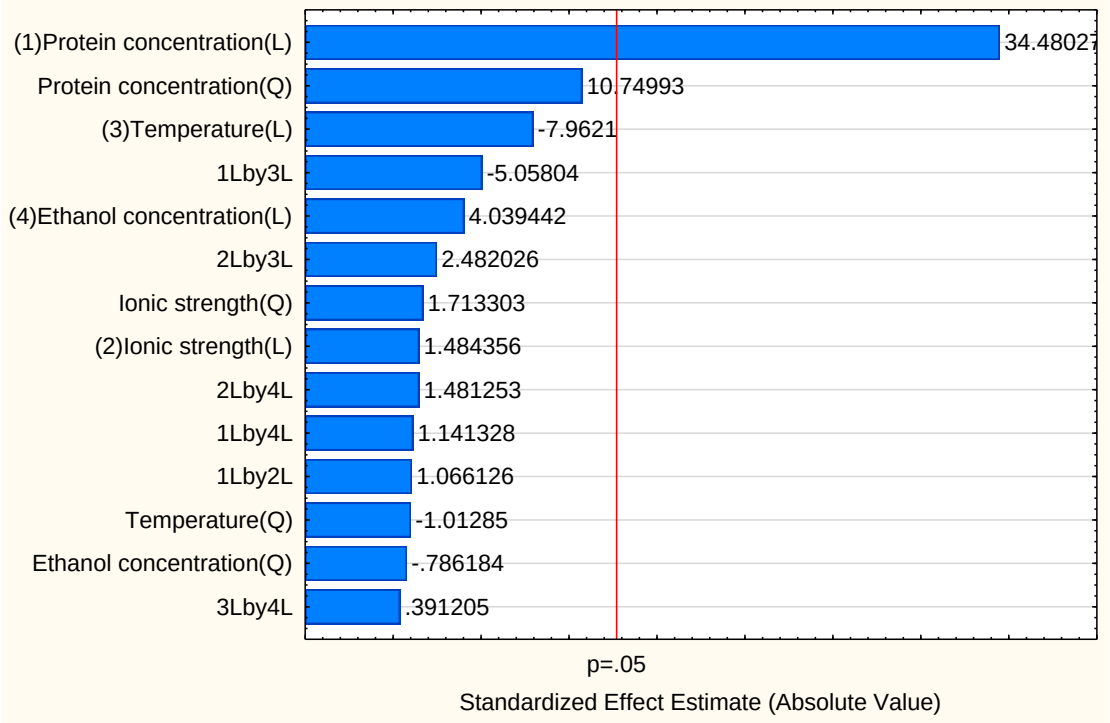


Figure 5:23: *Pareto chart of the standardized effects for process time of ultrafiltration, considering a standard batch of volume 1200L. Protein concentration had a statistically significant ($p < 0.05$) effect on process time.*

Table 5:21: *ANOVA table for process time of ultrafiltration; indicating the p-value, Beta co-efficient, t value and 95% confidence intervals.*

FACTOR	EFFECT	STD. ERR. PURE ERR	t (1)	P	COEFF	STD. ERR. COEFF	-95% CNF LMT	+95% CNF LMT
Adjusted R^2 = 86.31%								
Mean/Interc	3.94	0.16	24.44	0.026	3.94	0.16	1.89	5.99
(1) Protein concentration (L)	3.21	0.09	34.48	0.018	1.61	0.05	1.01	2.20
Protein concentration (Q)	1.17	0.11	10.75	0.059	0.59	0.05	-0.11	1.28
(2) Ionic strength (L)	0.14	0.09	1.48	0.377	0.07	0.05	-0.52	0.66
Ionic strength (Q)	0.19	0.11	1.71	0.336	0.09	0.05	-0.60	0.79
(3) Temperature(L)	-0.74	0.09	-7.96	0.080	-0.37	0.05	-0.96	0.22
Temperature(Q)	-0.11	0.11	-1.01	0.496	-0.06	0.05	-0.75	0.64
(4) Ethanol concentration (L)	0.38	0.09	4.04	0.154	0.19	0.05	-0.40	0.78
Ethanol concentration (Q)	-0.09	0.11	-0.79	0.576	-0.04	0.05	-0.74	0.65
1L by 2L	0.12	0.11	1.07	0.480	0.06	0.06	-0.66	0.79
1L by 3L	-0.58	0.11	-5.06	0.124	-0.29	0.06	-1.01	0.44
1L by 4L	0.13	0.11	1.14	0.458	0.07	0.06	-0.66	0.79
2L by 3L	0.28	0.11	2.48	0.244	0.14	0.06	-0.58	0.87
2L by 4L	0.17	0.11	1.48	0.378	0.08	0.06	-0.64	0.81
3L by 4L	0.04	0.11	0.39	0.763	0.02	0.06	-0.70	0.75

The Pareto chart (Figure 5:23) and the analysis of variance (Table 5:21) of the process time required to complete each experiment at prescribed factor levels reveals that only protein concentration (t = -34.48, p= 0.0019, 95% CI[2.20, 1.01]) has a statistically significant impact on process time during UF of HSA. The positive β co-efficient indicates that an increase in protein concentration results in a corresponding increase in process time. This is in good agreement with results confirmed for protein concentration which indicates that permeate flux decreases as protein concentration increases. The adjusted R^2 value suggests that at least 86.31% of the variability in process time is accounted for by taking into consideration protein concentration. The validity of the above conclusions are supported by the linearity of the normal probability plot vs. raw residuals (data not shown).

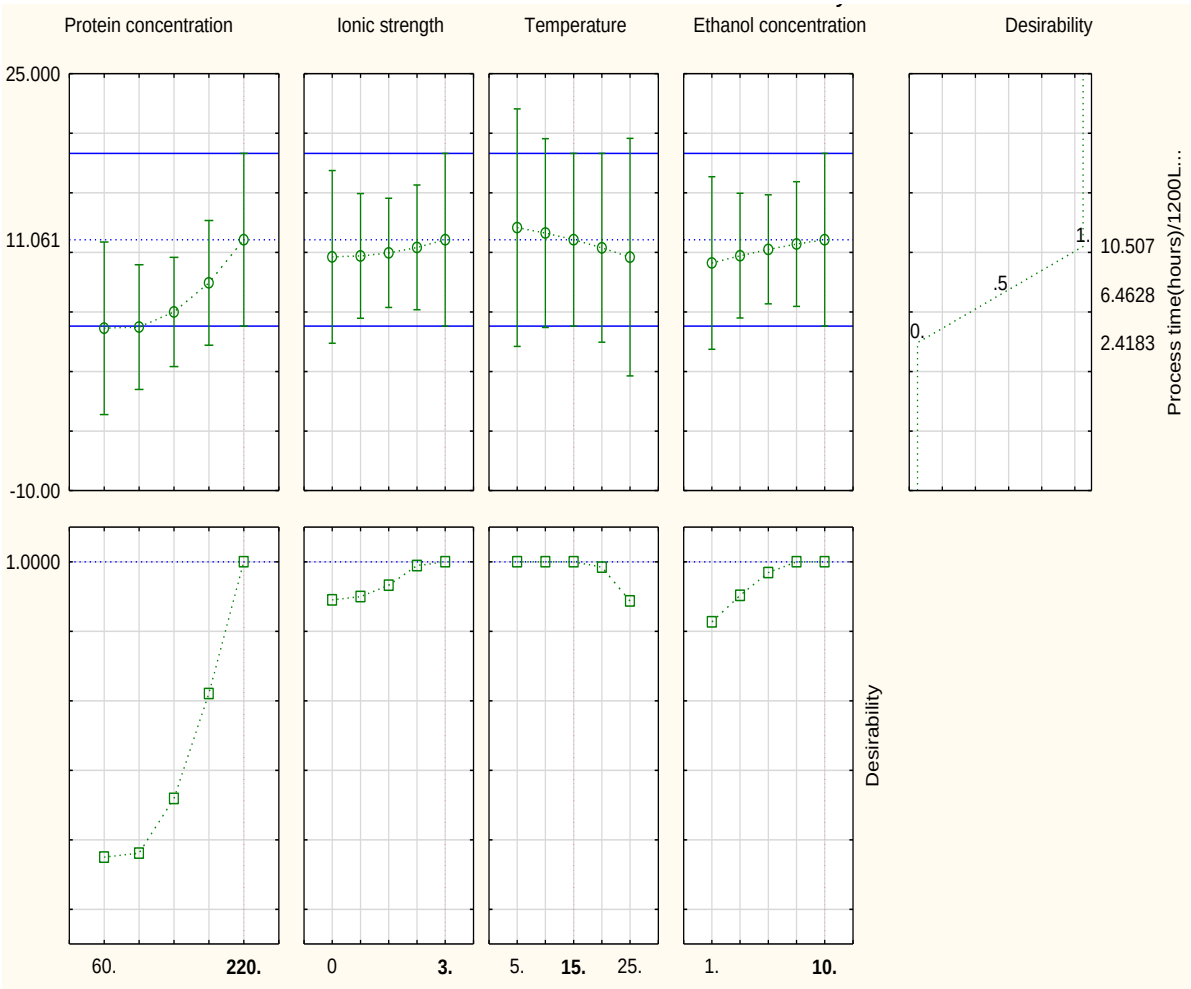


Figure 5:24: *Profile for predicted values and desirability for all factors for process time of ultrafiltration. The desirability function is maximized to achieve highest permeate flux for all factors, during the ultrafiltration of human serum albumin, were 1.000 is most desirable response and 0.000 is least desirable response. The predicted value for protein concentration that achieves the lowest process time is 60g/L and 100g/L.*

The profile for predicted value and desirability (Figure 5:37) indicates that a protein concentration within the range 60g/L to 100g/L gives the lowest process time during UF of HSA. This is in close agreement with the results obtained for protein concentration were the optimum protein concentration using the gel concentration model was determined to be approximately 100g/L.

Results presented in this study regarding the removal of unwanted solvents (ETOH) and micromolecules (e.g. potassium, aluminium and sodium) confirms that these solvents and salts are removed by the end of diafiltration step three. In an effort to decrease process cost, it is advised to determine the feasibility of completing diafiltration steps four and five WFI + 50% permeate or 100% permeate as diafiltration diluent.

6 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

The UF of HSA is a key unit operation with potential for optimization initiatives based on reducing capital expenditure and improving process efficiency with respect to minimizing CP and MF. This study was set out to evaluate the impact of protein concentration, ETOH concentration, ionic strength and temperature on permeate flux during the UF of HSA and to provide guidelines for the optimization of this unit operation through efficient process design and control strategies based on productivity and membrane performance. The impact of each factor, within specified levels, was determined for each step of UF of HSA. Key membrane performance indicators such as protein retention and permeate flux were also determined during experiments.

6.1.1 PROTEIN FEED CONCENTRATION

The UF of HSA is significantly impacted by protein concentration. During all experiments conducted, a steep decline in permeate flux was noted during the first concentration step, followed by quasi-steady state equilibrium during CVD. A further steep decline in permeate flux was noted during the second concentration step. These observations were in line with the theoretical and experimental evidence provided by several authors that outline the development of CP and MF during UF of HSA. It was further noted during this study that protein concentration does not have a significant impact on permeate flux during the first and second concentration steps, within the specified protein concentration range (60g/L to 220g/L), but it is the most significant factor that influences flux during CVD across diafiltration steps one to five. In general, as protein concentration increased, permeate flux during CVD decreased. This behaviour was explained through protein-protein interactions including the propensity for aggregation of proteins at high concentrations resulting in denaturation of proteins. Also the decline in permeate flux during CVD as protein concentration increased was also explained by protein-membrane interactions including protein adsorption, pore blocking and restrictions. Statistical analysis of the data showed that highest flux is obtained at the lowest (60g/L) protein concentration. This result was expected since the objectives of this study was related to maximizing permeate flux and therefore minimizing process time. The optimum protein concentration at which to complete UF of HSA was completed using the gel concentration model since the linear, inverse

dependence of permeate flux on protein concentration was established from the data accrued during this study. Further, this inverse dependence of permeate flux on protein concentration is attributed to CP and MF. The data from this study suggested a gel concentration (C_g) of 270g/L which correlates well with the C_g value (300g/L) for HSA UF established by Jaffrin and Charrier (1992). The optimum protein concentration was determined by dividing the C_g value of 270g/L by Napier's number (2.718) – the optimum protein concentration to complete UF of HSA according to the process defined by NBI is within the range 90g/L to 100g/L. The productivity analysis indicated that a minimal process time is achieved at a protein concentration within the range 60g/L to 100g/L. The protein concentration range for optimum productivity is in close agreement with the theoretical optimum protein concentration calculated using the gel concentration model. The productivity analysis coupled with the calculated optimum protein concentration range for UF of HSA suggests that the current batch size can be increased to approximately 276kg of Fraction V paste at the point of dissolution, corresponding to >30% increase in batch size.

6.1.2 ETHANOL CONCENTRATION

Ethanol is a polar solvent that is used extensively in the plasma fractionation industry as a precipitating agent. Ultrafiltration removes ETOH from the HSA solution to acceptable limits. Ethanol concentration had a statistically significant impact on permeate flux during diafiltration steps three to five and no significant effect on flux during the first and second concentration steps, within the levels of this factor selected for this study (1% - 10% v/v). Although the impact of ETOH during diafiltration steps three to five is considered statistically significant, the magnitude of the effect of ETOH is limited (<2.00LMH). The results from this study indicated that as ETOH concentration increased, permeate flux during UF of HSA decreased. Increased ETOH concentrations in protein feed solutions are known to induce structural and conformation changes to these proteins which results in an increase in the viscosity of the feed solution. Further, ETOH is known to have a dehydrating effect in WFI admixtures which causes precipitation of proteins. Increase in viscosity of feed solutions containing increased volumes of ETOH will result in decreasing the flow of solute through the membrane which results in low permeate flux during UF of HSA. Also, permeate flux during UF of protein solutions containing increased concentrations

of ETOH is low since the hydraulic permeability of the membrane is lowered due to the decrease in back-diffusion of protein molecules away from the protein-membrane interface resulting in a high concentration of protein molecules in the gel boundary layer.

According to this study, the ideal ETOH concentration that achieved optimum permeate flux during UF of HSA is within the range 0% to 7.75% (v/v). However, according to the fractionation process, the depth filtration step following the dissolution of Fraction V paste is completed at a process temperature of -3.5°C ± 0.2°C. A reduction of ETOH concentration during the dissolution of HSA has significant impact on the depth filtration unit operation since a reduction in ETOH concentration will increase the freezing point of the ETOH-WFI admixture and the process temperature for depth filtration will have to change from the current set point. Considering the requirement for the optimization of depth filtration, further validation requirements and most significantly the marginal negative effect of ETOH on permeate flux, it is proposed that the ETOH concentration remain unchanged (10% - v/v) until such time that the depth filtration process can be optimized.

6.1.3 TEMPERATURE

Temperature effects the physic-chemical properties of both the protein and membrane during UF and is therefore considered a key factor in the optimization of this unit operation. During this study temperature was varied within the range 5°C to 25°C and it was noted that this factor has a significant impact on permeate flux during CVD but not during first or second concentration steps, within the selected levels of this factor. The effect of temperature on permeate flux during UF of HSA is positive in that as temperature increases, permeate flux increases. The application of the Arrhenius Equation suggests that as temperature increases, viscosity decreases and according to Darcy's Law, the lower the viscosity of a solution the higher the flow through the membrane. Although the viscosity of HSA solutions during experiments was analytically determined, it is conferred from both Monkos (1996) and Masuelli (2013) that the viscosity of BSA solutions of varying concentrations, decreases as temperature is increased. Therefore it was concluded that the low viscosity of the HSA solution coupled with the efficient back diffusion of protein molecules away from the protein-

membrane interface resulted in reducing the concentration of protein in the gel boundary layer which caused an increase in permeate flux as temperature was increased. According to the results obtained in this study, the optimum permeate flux is achieved at the temperature set point of 25°C with marginally lower permeate flux achieved at 20°C. HSA is known to undergo reversible conformational and structural changes within certain temperature ranges; beyond a defined limit temperature of 45°C; beyond this temperature these changes become irreversible. It is well established that HSA is stable in the native conformation at temperatures below 30°C. It is recommended that the process temperature for the UF of HSA remain at the current set point of 20°C ± 2°C since this set point is in close agreement with the optimum temperature set point recommended by this study.

6.1.4 pH AND IONIC STRENGTH

During this study, pH of the test solutions was maintained at pH 7.2 ± 0.2 for all experiments completed since this physiological pH maintains the optimum stability of the HSA protein in the native state. Several studies have confirmed that the permeate flux during UF of proteins is at its lowest when completed at a pH close to the IEP of the protein being ultrafiltered; the selected pH for this study is further away from the IEP thus ensuring that pH does not inhibit permeate flux during UF of HSA.

Ionic strength had no significant effect on permeate flux during first, second concentration step and diafiltration steps one to three, within the levels of the factor selected for this study (1M to 3M). Ionic strength only had a statistically significant effect on permeate flux during diafiltration step five, whilst a substantive significant effect was noted during diafiltration step four. The effect of ionic strength on permeate flux was negative in that an increase in ionic strength resulted in a decrease in permeate flux. These results were extensively explained by the DLVO theory and double layer interactions of molecules whereby NaCl acts as an electrostatic shield which reduces the overall net charge of the protein molecule thus increasing protein adsorption to the membrane surface. This results in decrease of permeate flux during UF of HSA. These results indicated that NaCl ions only have a significant impact on permeate flux during UF of HSA when all other ions e.g. aluminium and citrate and polar solvents e.g. ETOH are removed from the solution. This clearly suggests that when the competition for binding localities on the HSA molecule is reduced, NaCl molecules bind to the regions

and initiate the electrostatic shielding effects described herein; ultimately resulting in a decrease in permeate flux during UF of HSA.

Considering the negative impact of ionic strength on permeate flux during the UF of HSA, as confirmed by this study, it is recommended that the current NBI process continue to use WFI as buffer during CVD.

6.2 RECOMENDATIONS

The key goal of this study has been clearly stated and can be summarized as the optimization of the factors that influence UF of HSA. The recommended process changes based on the evidence provided in this study for a starting batch volume of 1150L is as follows:

- Maintain process temperature at 20°C ± 2°C.
- The protein concentration during depth filtration is the process limiting step and this concentration is set at 60g/L. Therefore dissolve 276kg of Fraction V paste in 1150L WFI containing 10% ETOH (v/v).
- Complete first concentration step of the depth filtered HSA solution from 60g/L (~1150L) to approximately 100g/L ± 10g/L (target volume approximately 694.58L).
- Complete CVD using WFI as the diafiltration diluent to maintain constant volume.
- Complete second concentration step to 210g/L, before formulation.

Analytical test results indicate that ETOH and salts are removed to acceptable limits by the end of diafiltration step three. It is recommended to complete a cost benefit analysis to determine the impact of using 50% permeate + 50% WFI or 100% permeate as the diafiltration diluent for diafiltration steps four and five. This strategy may save money when compared to using WFI only as a diafiltration diluent during diafiltration steps four and five.

Ultrafiltration is a key membrane technology that is influenced by several factors and also allows for significant optimization initiatives. The various steps of UF of HSA is

dependent on various factors, however, protein concentration is the most significant of these factors. Protein concentration has a direct impact on CP and MF, the effects of which are demonstrated through key membrane performance characteristics such as permeate flux, protein retention and membrane flux recovery. This study has confirmed the optimum protein concentration at which to complete UF of HSA. Further, the benefits of reducing ETOH concentration, reducing temperature or using an ionic solution is not feasible for the current process and is heavily reliant on optimization of related upstream unit operations, particularly depth filtration.

The aims and objectives of this study have been achieved, however, future work should include the effect of different membrane types (e.g. ceramic membranes) on permeate flux during the UF of HSA and feasibility of incorporating a pulsation module to improve permeate flux during UF of HSA. Future research should also consider developing a dynamic ultrafiltration model for this process. Lastly, one may consider a review and optimization initiative with respect to the upstream process of depth filtration and the impact (process optimization and regulatory compliance) of reducing ETOH concentration on depth filtration. An optimization study on depth filtration, especially with respect to the effects of ETOH, will have an effect on the downstream process of UF of HSA.

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8 APPENDIX A

Specification, performance data and quality certificate for T-series membrane cassette.

A.1. Specifications and performance data for T-series membrane cassette.

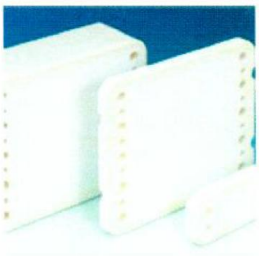
Pall Corporation

Contact Us: www.pall.com/contact

T-Series Membrane Cassettes

Description

High Performance Omega PES Membrane Combined with Significant Material and Design Improvements Deliver Highly Reliable Fluid Dynamics and Better Process Economics



- **Easy implementation into your process**. The new T-series cassettes offer significant improvements in construction and design while utilizing the same proven Omega polyethersulfone (PES) membrane to reduce revalidation requirements.
- **Increased safety, reliability, and reproducibility**. Superior new materials of construction are durable and stable, exhibit very low extractables, and offer broad chemical compatibility.
- **High flux, high selectivity, and low protein binding** are achieved through the use of Omega PES membrane.
- **Improved process performance**. Cassettes are designed to provide optimal mass transfer to improve your process economics.
- **Easy scale up for robust purification processes.** Available in scalable formats with the same materials of construction from development to production-scale processes.
- **Enhanced validation**. T-series cassettes surpass the latest biopharmaceutical and regulatory standards such as biological reactivity, extractables, and TOC. Product claims are backed by extensive validation documentation to support the use of T-Series cassettes in your process.

Proven Omega Membrane

Pall's Omega polyethersulfone (PES) membranes offer high flux and selectivities. They have been specifically modified to minimize protein binding to the surface and interstitial structure of the membrane. This polymeric membrane is stable against biological and physical degradation due to the unique chemical properties of PES.

Omega membranes are cast on a highly porous, non-woven polyolefin support. They have an anisotropic structure, a thin skin-like top layer with a highly porous underlying support. The structure of the skin determines the porosity and permeability characteristics of the membrane and can typically be cleaned quicker and easier than membranes with a uniform, sub-micron depth structure. This membrane is compatible with acids, bases and a variety of other cleaning agents. Omega membranes are available in a wide range of nominal molecular weight cutoffs (NMWC).

The T-Series Advantage

Superior new materials of construction and improved cassette design increase process safety, reliability, reproducibility, and productivity:

- The feed and permeate screen material for T-Series cassettes is made from polypropylene which is highly resistant to sodium hydroxide.
- Larger feed and permeate ports provide lower pressure drops.
- T-Series cassettes have been designed to provide maximum mass transfer through the membrane, resulting in faster processing times or reduced area installations compared to existing similar cassette formats.

Application

T-Series cassettes with Omega membrane are designed for development, pilot, and production-scale TFF applications in diverse biological and biopharmaceutical processes. They are especially useful in:

- Vaccine and conjugate concentration and diafiltration
- Purification and recovery of monoclonal antibodies (mAb) or recombinant proteins
- Blood plasma fractionation and purification

Product Platforms

T-Series cassettes complement Pall's highly successful Centramate™ and Centrasette™ products, and have been developed following years of experience designing and optimizing our customers' biotech and biopharmaceutical processes.

T-Series cassettes for Centramate holders are offered in scalable membrane formats with effective filtration areas (EFA) from as low as 200 cm^2 (0.215 ft^2) to 0.5 m^2 (5.4 ft^2), making them ideal for process development and small-scale production of 0.1 L to 125 L. Centrasette T-Series cassettes, in combination with Centrasette holders, can process thousands of liters with installations incorporating hundreds of square meters of EFA.

Specifications

Materials of Construction

Membrane	Omega polyethersulfone
Support	Polyolefin
Screens	Polypropylene
Encapsulent	Polyurethane with white pigment (TiO_2)
Permeate Seals	Platinum cured silicone
Gaskets	Medical grade, platinum cured silicone

Operating Limits

Maximum Pressure[1]	6 barg (90 psig) @ 23 °C 4 barg (60 psig) @ 55 °C
Maximum TMP	4 barg (60 psig) @ 55 °C
Temperature Range[2]	-5 – 55 °C
pH Range	2 – 14

[1]*Pressure rating will be dependant on rating of the lowest system component.*
[2]*Cassettes must not be allowed to freeze.*

Typical Operating Parameters

Cross Flow Rate for Processing	5 – 7 L/min/m^2 (0.5 – 0.7 L/min/ft^2)
Cross Flow Rate for Cleaning	8 – 10 L/min/m^2 (0.8 – 1.0 L/min/ft^2)

Integrity Test

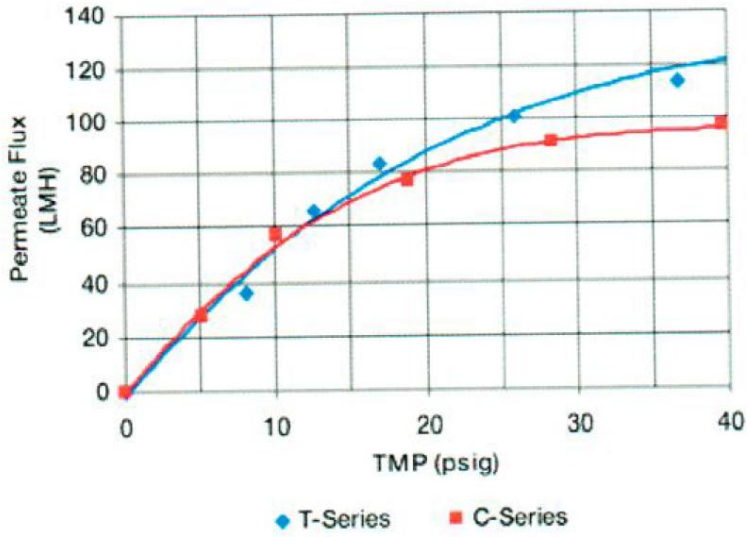
Test Pressure	2 barg (30 psig)
Maximum Air Forward Flow	< 1600 sccm/m^2 (< 150 sccm/ft^2)

Shelf Life
The shelf life of cassettes packaged in 0.3 N sodium hydroxide is expected to be three years from the date of manufacture when the cassettes are stored unopened in the original packaging at 4 – 25 °C and protected from direct light. Extended shelf life studies are ongoing.

Biological Safety
All of the materials of construction in the T-Series cassettes have been tested and meet requirements for United States Pharmacopeia (USP) Biological Reactivity Test, *In Vivo* <88> at 70 °C.

Performance

Figure 1
Omega 10 kD Membrane, Centramate C vs. T-Series Membrane Cassettes at a 5 L/min/m^2Cross Flow Rate



Documentation

Each T-Series membrane cassette has a unique serial number for full traceability. Each cassette is supplied with:

- Certificate of Quality
- Membrane Cassette Care and Use Procedures
- Material Safety Data Sheet (MSDS) for cassette preservative
- Two platinum cured silicone gaskets

The full Validation Guide is available on request from your local Pall contact. Pall also offers a comprehensive Validation Service for specific tests (such as compatibility) in your process fluid. As always, Pall's Downstream Processing Specialists are available to train and support you in the optimization of your TFF processes.

A.2. Quality certificate for T-series membrane cassette.

Pall Corporation

Certificate of Quality

We hereby certify that

Pall® : **10K OMEGA CENTRAMATE T-SERIES 0.1m² / 1.1ft²**

Part Number: **OS010T12**

Serial Number: **34234011R**

Membrane Lot Number: **H4191A**

was manufactured in a controlled environment.

Materials of Construction

Conformance to Regulatory Requirements

This product may be used in conjunction with current good manufacturing practices as per Title 21 of the **Code of Federal Regulations** (CFR) parts 210 and 211.72.

This product does not contain materials of construction that are considered TSE or BSE risk materials according to current legislation and guidelines (reference European CPMP EMA/410/01 and **Code of Federal Regulations**, Title 21 Part 189.5).

Bio Safety Data

The fluid path components have met the specifications for biological tests listed in the current revision of the **United States Pharmacopeia** (USP) for Class VI plastics. Contact Pall for further information regarding materials of construction.

Cassette Validation

During validation, cassettes were tested using recommended care and use procedures to establish suitibility with respect to the following characteristics:

- Residual TOC
- Bacterial Endotoxin Levels
- Nonvolatile Extractable Residue

Product Quality

Membrane Quality

Representative membrane samples from this manufacturing lot underwent the following tests and the lot was released by Quality Control when it was verified that their respective criteria were met:

- Water Permeability
- Solute Marker Passage

Integrity

This product successfully passed a final integrity test based on an air diffusion / forward flow test that ensures membrane and seal integrity.

Recommended Storage Conditions

This Pall cassette can be expected to perform within specifications if stored and handled in a manner consistent with the parameters below:

- The cassette is stored unopened in the original packaging at 4-25°C, and in a dry environment.
- The cassette is protected from direct sunlight, radiation or weather conditions.
- Care is taken to avoid physical damage while handling.
- Thermal shock is avoided.

In addition to the above tests, this product met manufacturing inspection standards and requirements for full traceability in an ISO 9001 certified facility. **These products are not supplied sterile.** Users should test the membrane integrity prior to use. Consider only unopened, undamaged packages for use. Further information is available by contacting Pall.

Moira Bilich, Quality Manager, Pall Hauppauge
225 Marcus Boulevard, Hauppauge, New York 11788

22/August/2014

Date of Manufacture

CoQ0010E rev 04

www.pall.com

Filtration. Separation. Solution. SM

9 APPENDIX B

Protocol for preparing start protein feed material for ultrafiltration experiments i.e. dissolution and depth filtration of Fraction V paste (containing HSA)

NATIONAL BIOPRODUCTS INSTITUTE

PROCESS ENGINEERING AND DEVELOPMENT

LABORATORY PROTOCOL: DISSOLUTION AND DEPTH FILTRATION OF FRACTION V PASTE

National Bioproducts Institute
10 Eden Road
Pinetown,3600
www.nbi-kzn.org.za
Phone: (031) 719-6789

REVISION HISTORY

Rev.	Date	Developed By:	Revision Summary
0	12/02/2014	B. Bhiman	**Draft 01**
1	15/03/2014	B. Bhiman	**Final Version**
2			
3			

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1. INTRODUCTION

Fraction V paste is fractionated from human plasma using the techniques described by Kistler and Nitschmann (1962). Fraction V paste is the active pharmaceutical ingredient (API) containing human serum albumin (HSA). The experiments related to optimization of the ultrafiltration of HSA requires the preparation and depth filtration of Fraction V paste according to current NBI manufacturing guidelines which is appropriately downscaled to fit the laboratory scale. The fractionation of Fraction V paste from starting plasma is described elsewhere; Fraction V paste produced by NBI was used in all preparations of HSA starting materials used for ultrafiltration experiments. A mass of Fraction V paste was dissolved in water for injection (WFI); as per current NBI procedures but proportionately scaled down to suit the laboratory applications. However, the paste was not dissolved in 10% ethanol (96% purity; v/v) since ethanol is a key factor being tested during these optimization experiments. Therefore, ethanol (96% purity; v/v) was added in volumes according to the factor level concentrations required per experiment. Depth filtration was completed using a laboratory type depth filter and an AF9 (filter pad used in current manufacturing process) filter pad. Since ethanol was not added to the dissolved Fraction V paste (as is done in current manufacturing process); depth filtration was completed at the dissolution temperature of 4°C ± 1°C. This dissolved and depth filtered Fraction V paste is tested for protein concentration and ethanol concentration using the Cobas Fara method and GC method respectively. Appropriate volumes of the HSA solution for each experiment is determined according to the protein concentration required for that experiment. Ethanol (96% - v/v) is added according to the concentration required for each experiment and is based on the starting volume of HSA solution used for that experiment.

2. OBJECTIVE

The objective of this protocol is to confirm the procedure for the laboratory scale preparation of dissolved and depth filtered Fraction V paste. This protocol refers to the dissolution of Fraction V paste, the depth filtration of dissolved Fraction V paste, related pH adjustments and volumetric adjustment to produce a human serum albumin (HSA) solution of approximately 5L volume with protein concentration within the range 40-60g/L protein concentration, pH 7.2.

3. ABBREVIATIONS

HSA – Human serum albumin
WFI – Water for injection
ETOH – Ethanol
NaOH – Sodium hydroxide
RO – Reverse osmosis
EDTA – Ethylene-di-amine-tetra-acetic Acid

API – Active pharmaceutical ingredient

4. EQUIPMENT LIST

- 5L, 2L plastic beakers
- 50mL, 100mL measuring cylinders
- Mechanical stirrer
- Thermometer
- pH meter
- Balance

5. MATERIALS LIST

- Water for Injection (WFI)
- Fraction V paste
- 2M NaOH
- 2M HCL
- 0.67M Ehtylenediaminetetracetic Acid (EDTA)
- 96% (v/v) ETOH

6. METHODOLOGY

6.1 Complete the relevant section of Appendix A, for all steps in this methodology section.

6.2 Prepare 2M NaOH. Complete Appendix B

6.3 Prepare 0.67M EDTA. Complete Appendix B

6.4 Request 2.5kg of Fraction V paste from the dispensary team. Note the batch number of the Fraction V paste; dispensary pharmacist/team leader to authorise release of paste. Complete Appendix A, 1.

6.5 Collect approximately 5.0L of WFI in a 5.0L plastic beaker and cool to 4°C ± 1°C. Check the temperature of the WFI periodically. Ensure the temperature of the WFI is 4°C ± 1°C **BEFORE** addition of Fraction V paste. Complete Appendix A, 2.

6.6 Add Fraction V paste of mass 1.20kg ± 0.2kg to approximately 3L of cooled WFI in a clean 5L beaker. Note: weigh the plastic bag containing Fraction V paste **BEFORE** dispensing paste into beaker. Thereafter weigh the plastic bag **AFTER** dispensing paste into beaker. Complete Appendix A, 3.

6.7 Assemble the mechanical stirrer and place into the beaker containing the Fraction V paste in WFI. Set the stirrer to speed of 200rpm and start the dissolution of the Fraction V paste. Dissolution process to be completed at 4°C ± 1°C. Complete Appendix A, 4.

6.8 Allow the solution to continuously stir for not less than 8 hours. Thereafter, switch stirrer off and make solution up to approximately 5L using the rest of the cooled WFI. Complete Appendix A, 5.

6.9 Allow the dissolved Fraction V paste to settle for not less than 4 hours.

6.10 Check and adjust pH to pH 4.57 ± 0.03 using 2M NaOH or 2M HCL. Complete Appendix A, 6.

6.11 Assemble the laboratory type depth filtration module with 1 x AF9 depth filter pad. Ensure that the screws are sufficiently tightened to prevent leakage of Fraction V paste solution during depth filtration.

6.12 Depth filter the solution using clean compressed air. Maintain a volumetric flow rate of less than 125mL/min during depth filtration. Complete Appendix A, 7.

6.13 Test the pH of the solution and adjust the pH to pH 7.25 ± 0.1 using 2M NaOH. Complete Appendix A, 8.

6.14 Add 88ml of 0.67M EDTA to this solution. Complete Appendix A, 9.

6.15 Test the pH of the solution again and adjust the pH to pH 7.25 ± 0.1 using 2M HCL. Complete Appendix A, 10.

APPENDIX A

1. REQUISITION OF FRACTION V PASTE

	DATE	BATCH NO	RELEASE SIGN	PROC ENG SIGN	MASS OF PASTE (g)
Requisition of Fraction V paste					

2. WATER COLLECTION

	DATE	VOLUME (L)	INITIAL TEMP (°C)	TIME	FINAL TEMP (°C)	TIME	PROC ENG SIGN
Collect 4.8L WFI – Beaker 1							
Collect 4.8L WFI – Beaker 2							

3. FRACTION V PASTE ADDITION TO WFI

	DATE	MASS OF PASTE (g)	VOLUME OF WFI (L)	TIME	PROC ENG SIGN
Addition of FV paste to WFI – Beaker 1					
Addition of FV paste to WFI – Beaker 2					

4. DISSOLUTION

	DATE	TIME START	TIME END	STIRRER SPEED	PROC ENG SIGN
Dissolution of Fraction V paste and stir - Beaker 1					
Dissolution of Fraction V paste and stir - Beaker 2					

5. DILUTION TO ~5.0L

	DATE	FINAL VOLUME OF DISSOLVED HSA SOLUTION (L)	PROC ENG SIGN
Dilute solution to ~5.0L using WFI - Beaker 1			
Dilute solution to ~5.0L using WFI - Beaker 2			

6. TESTING AND CHANGING pH – 4.57 ± 0.03

Beaker 1	DATE	INITIAL pH	AMOUNT HCL ADDED	FINAL pH	PROC ENG SIGN
Changing the pH of the solution to pH 4.57 ± 0.03 using 2M NaOH/HCL					

Beaker 2	DATE	INITIAL pH	AMOUNT HCL ADDED	FINAL pH	PROC ENG SIGN
Changing the pH of the solution to pH 4.57 ± 0.03 using 2M NaOH/HCL					

7. DEPTH FILTRATION

	DATE	TIME START	TIME END	PROC ENG SIGN
Depth filtration using AF9 filter pad – Beaker 1				
Depth filtration using AF9 filter pad – Beaker 2				

8. TESTING AND CHANGING pH – 7.25 ± 0.1

Beaker 1	DATE	INITIAL pH	AMOUNT HCL ADDED	FINAL pH	PROC ENG SIGN
Changing the pH of the solution to pH 7.25 ± 0.1 using 2M NaOH					

Beaker 2	DATE	INITIAL pH	AMOUNT HCL ADDED	FINAL pH	PROC ENG SIGN
Changing the pH of the solution to pH 7.25 ± 0.1 using 2M NaOH					

9. EDTA ADDITION

	DATE	AMOUNT OF EDTA ADDED	PROC ENG SIGN
EDTA ADDITION			

10. TESTING AND CHANGING pH – 7.25 ± 0.1

Beaker 1	DATE	INITIAL pH	AMOUNT HCL ADDED	FINAL pH	PROC ENG SIGN
Changing the pH of the solution to pH 7.25 ± 0.1using 2M HCL					

Beaker 2	DATE	INITIAL pH	AMOUNT HCL ADDED	FINAL pH	PROC ENG SIGN
Changing the pH of the solution to pH 7.25 ± 0.1 using 2M HCL					

APPENDIX B: REAGENT PREPARATION

1. **Reagent:** _____

2. **Date of preparation:** _____

3. **Prepared by:** _____

4. **Preparation:**

   Concentration (C):_____

   Molar mass (Mr):_____

   Volume (V):_____

   Mass of reagent required = C x Mr x V

5. **Sign Off**

_____ _____

Process Engineer/Scientist Date

10 APPENDIX C

Experimental data (nominal water permeability, permeate flux) for experiments completed as part of this study.

28-Mar-15	
Experiment 1	
Start UF Sample No	1028727

	REQUIRED	EXPERIMENTAL	VARIANCE
Protein concentration (g/L)	100.00	9.29	-0.71
Ionic strength (M)	0.75	0.75	0.00
Temperature (°C)	10.00	11	1.00
Ethanol Concentration (%)	3.25	2.85	-0.40
TMP (Bar)	1.00	1.15	0.15

CLEAN WATER FLUX

Hz	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	PERMEATE FLUX 20°c	TEMPERATURE	NWP (LMH/PSI)	NWP20°c (LMH/PSI)
4.00	5.00	0.00	0.00	2.50	0.17	1.00	100.00	6.00	0.10	60.00	52.80	25.50	12.00	10.56
5.00	10.00	0.00	0.00	5.00	0.34	1.00	155.00	9.30	0.10	93.00	81.84	25.50	9.30	8.18
5.80	15.00	0.00	0.00	7.50	0.51	1.00	195.00	11.70	0.10	117.00	102.96	25.50	7.80	6.86
6.30	20.00	0.00	0.00	10.00	0.68	1.00	245.00	14.70	0.10	147.00	129.36	25.50	7.35	6.47

FIRST CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.90	0.00		22.50	9.50	0.00	16.00	1.09	1.00	75.74	4.54	0.10	45.44	10.00
									75.74				

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.80	0.00	1.00	23.50	9.00	0.00	16.25	1.11	1.00	72.52	4.35	0.10	43.51	11.00
3.80	5.00	1.00	24.00	9.50	0.00	16.75	1.14	1.00	76.44	4.59	0.10	45.86	11.00
3.80	11.00	2.00	24.50	9.50	0.00	17.00	1.16	1.00	76.44	4.59	0.10	45.86	10.50
3.80	15.00	2.00	24.50	9.50	0.00	17.00	1.16	1.00	76.44	4.59	0.10	45.86	10.50
3.80	22.00	3.00	24.50	9.50	0.00	17.00	1.16	1.00	76.44	4.59	0.10	45.86	10.00
3.80	29.00	3.00	24.50	9.50	0.00	17.00	1.16	1.00	76.44	4.59	0.10	45.86	10.00
3.80	35.00	4.00	24.50	9.50	0.00	17.00	1.16	1.00	78.40	4.70	0.10	47.04	10.50
3.80	40.00	4.00	24.50	9.50	0.00	17.00	1.16	1.00	77.42	4.65	0.10	46.45	10.50
3.80	45.00	5.00	24.50	9.50	0.00	17.00	1.16	1.00	78.40	4.70	0.10	47.04	10.50
3.80	50.00	5.00	24.50	9.50	0.00	17.00	1.16	1.00	77.42	4.65	0.10	46.45	11.00
							1.15		76.64			45.98	10.55

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.80	0.00	1.00	23.50	9.00	0.00	16.25	1.11	1.00	72.52	4.35	0.10	43.51	11.00
3.80	5.00	1.00	24.00	9.50	0.00	16.75	1.14	1.00	76.44	4.59	0.10	45.86	11.00
												43.51	
3.80	11.00	2.00	24.50	9.50	0.00	17.00	1.16	1.00	76.44	4.59	0.10	45.86	10.50
3.80	15.00	2.00	24.50	9.50	0.00	17.00	1.16	1.00	76.44	4.59	0.10	45.86	10.50
												45.86	
3.80	22.00	3.00	24.50	9.50	0.00	17.00	1.16	1.00	76.44	4.59	0.10	45.86	10.00
3.80	29.00	3.00	24.50	9.50	0.00	17.00	1.16	1.00	76.44	4.59	0.10	45.86	10.00
												45.86	
3.80	35.00	4.00	24.50	9.50	0.00	17.00	1.16	1.00	78.40	4.70	0.10	47.04	10.50
3.80	40.00	4.00	24.50	9.50	0.00	17.00	1.16	1.00	77.42	4.65	0.10	46.45	10.50
												47.04	
3.80	45.00	5.00	24.50	9.50	0.00	17.00	1.16	1.00	78.40	4.70	0.10	47.04	10.50
3.80	50.00	5.00	24.50	9.50	0.00	17.00	1.16	1.00	77.42	4.65	0.10	46.45	11.00
							1.15		76.64			47.04	10.55

SECOND CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.80			24.50	9.50	0.00	17.00	1.16	1.00	63.55	3.81	0.10	38.13	11.00

02-Mar-15	
Experiment 2	
Start UF Sample No	1028728

	REQUIRED	EXPERIMENTAL	VARIANCE
Protein concentration (g/L)	100.00	8.45	-1.55
Ionic strength (M)	0.75	0.75	0.00
Temperature (°C)	10.00	11.5	1.50
Ethanol Concentration (%)	7.75	6.24	-1.51
TMP (Bar)	1.00	1.1	0.10

CLEAN WATER FLUX

Hz	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	PERMEATE FLUX 20°c	TEMPERATURE	NWP (LMH/PSI)	NWP20°c (LMH/PSI)
4.30	5.00	0.00	0.00	2.50	0.17	1.00	100.00	6.00	0.10	60.00	49.80	28.00	12.00	9.96
5.50	10.00	0.00	0.00	5.00	0.34	1.00	160.00	9.60	0.10	96.00	79.68	28.00	9.60	7.97
6.00	15.00	0.00	0.00	7.50	0.51	1.00	195.00	11.70	0.10	117.00	97.11	28.00	7.80	6.47
6.90	20.00	0.00	0.00	10.00	0.68	1.00	240.00	14.40	0.10	144.00	119.52	28.00	7.20	5.98

FIRST CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.30	0.00		24.00	10.00	0.00	17.00	1.16	1.00	54.29	3.26	0.10	32.57	10.00

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.10	0.00	1.00	22.00	9.50	0.00	15.75	1.07	1.00	55.86	3.35	0.10	33.52	11.00
3.20	5.00	1.00	23.00	10.00	0.00	16.50	1.12	1.00	60.76	3.65	0.10	36.46	11.50
3.20	10.00	1.00	23.00	10.00	0.00	16.50	1.12	1.00	60.76	3.65	0.10	36.46	11.50
3.20	15.00	2.00	22.00	9.50	0.00	15.75	1.07	1.00	61.74	3.70	0.10	37.04	11.50
3.20	20.00	2.00	22.00	9.50	0.00	15.75	1.07	1.00	62.72	3.76	0.10	37.63	11.00
3.20	25.00	2.00	22.00	9.50	0.00	15.75	1.07	1.00	61.74	3.70	0.10	37.04	11.00
3.30	30.00	3.00	22.50	10.00	0.00	16.25	1.11	1.00	64.68	3.88	0.10	38.81	11.50
3.30	35.00	3.00	22.50	10.00	0.00	16.25	1.11	1.00	64.68	3.88	0.10	38.81	11.00
3.30	40.00	3.00	22.50	10.00	0.00	16.25	1.11	1.00	64.68	3.88	0.10	38.81	10.50
3.30	45.00	4.00	22.50	10.00	0.00	16.25	1.11	1.00	64.68	3.88	0.10	38.81	10.50
3.30	50.00	4.00	22.50	10.00	0.00	16.25	1.11	1.00	64.68	3.88	0.10	38.81	10.00
3.30	55.00	5.00	22.50	10.00	0.00	16.25	1.11	1.00	64.68	3.88	0.10	38.81	10.00
3.30	60.00	5.00	22.50	10.00	0.00	16.25	1.11	1.00	64.68	3.88	0.10	38.81	10.00
3.30	65.00	5.00	22.50	10.00	0.00	16.25	1.11	1.00	64.68	3.88	0.10	38.81	10.50
							1.10		62.93			37.76	10.82

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.10	0.00	1.00	22.00	9.50	0.00	15.75	1.07	1.00	55.86	3.35	0.10	33.52	11.00
3.20	5.00	1.00	23.00	10.00	0.00	16.50	1.12	1.00	60.76	3.65	0.10	36.46	11.50
3.20	10.00	1.00	23.00	10.00	0.00	16.50	1.12	1.00	60.76	3.65	0.10	36.46	11.50
												35.48	
3.20	15.00	2.00	22.00	9.50	0.00	15.75	1.07	1.00	61.74	3.70	0.10	37.04	11.50
3.20	20.00	2.00	22.00	9.50	0.00	15.75	1.07	1.00	62.72	3.76	0.10	37.63	11.00
3.20	25.00	2.00	22.00	9.50	0.00	15.75	1.07	1.00	61.74	3.70	0.10	37.04	11.00
												37.24	
3.30	30.00	3.00	22.50	10.00	0.00	16.25	1.11	1.00	64.68	3.88	0.10	38.81	11.50
3.30	35.00	3.00	22.50	10.00	0.00	16.25	1.11	1.00	64.68	3.88	0.10	38.81	11.00
3.30	40.00	3.00	22.50	10.00	0.00	16.25	1.11	1.00	64.68	3.88	0.10	38.81	10.50
												38.81	
3.30	45.00	4.00	22.50	10.00	0.00	16.25	1.11	1.00	64.68	3.88	0.10	38.81	10.50
3.30	50.00	4.00	22.50	10.00	0.00	16.25	1.11	1.00	64.68	3.88	0.10	38.81	10.50
												38.81	
3.30	55.00	5.00	22.50	10.00	0.00	16.25	1.11	1.00	64.68	3.88	0.10	38.81	10.50
3.30	60.00	5.00	22.50	10.00	0.00	16.25	1.11	1.00	64.68	3.88	0.10	38.81	10.50
3.30	65.00	5.00	22.50	10.00	0.00	16.25	1.11	1.00	64.68	3.88	0.10	38.81	10.50
												38.81	

SECOND CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.30	0.00		22.50	10.00	0.00	16.25	1.11	1.00	58.81	3.53	0.10	35.29	10.00

01-Apr-15	
Experiment 3	
Start UF Sample No	1028729

	REQUIRED	EXPERIMENTAL	VARIANCE
Protein concentration (g/L)	100.00	10.33	0.33
Ionic strength (M)	0.75	0.75	0.00
Temperature (°C)	20.00	21	1.00
Ethanol Concentration (%)	3.25	2.63	-0.62
TMP (Bar)	1.00		-1.00

CLEAN WATER FLUX

Hz	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	PERMEATE FLUX 20°c	TEMPERATURE	NWP (LMH/PSI)	NWP20°c (LMH/PSI)
3.70	5.00	0.00	0.00	2.50	0.17	1.00	85.00	5.10	0.10	51.00	47.43	23.00	10.20	9.49
4.60	10.00	0.00	0.00	5.00	0.34	1.00	135.00	8.10	0.10	81.00	75.33	23.00	8.10	7.53
5.30	15.00	0.00	0.00	7.50	0.51	1.00	180.00	10.80	0.10	108.00	100.44	23.00	7.20	6.70
5.70	20.00	0.00	0.00	10.00	0.68	1.00	220.00	13.20	0.10	132.00	122.76	23.00	6.60	6.14

FIRST CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.20	0.00		23.00	10.50	0.00	16.75	1.14	1.00	90.16	5.41	0.10	54.10	10.50
3.20	0.00		25.00	11.50	0.00	18.25	1.24	1.00	87.22	5.23	0.10	52.33	10.50
3.00	0.00		24.00	10.50	0.00	17.25	1.17	1.00	70.56	4.23	0.10	42.34	10.50
2.80	0.00		25.00	10.00	0.00	17.50	1.19	1.00	60.76	3.65	0.10	36.46	10.50
									77.18			46.31	

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.80	0.00	1.00	23.50	9.50	0.00	16.50	1.12	1.00	58.80	3.53	0.10	35.28	19.00
2.90	5.00	1.00	23.50	10.50	0.00	17.00	1.16	1.00	64.68	3.88	0.10	38.81	19.00
3.00	10.00	1.00	23.50	10.50	0.00	17.00	1.16	1.00	73.50	4.41	0.10	44.10	19.50
3.00	15.00	2.00	23.50	10.50	0.00	17.00	1.16	1.00	72.52	4.35	0.10	43.51	19.50
3.00	20.00	2.00	23.50	10.50	0.00	17.00	1.16	1.00	73.50	4.41	0.10	44.10	20.00
3.00	25.00	3.00	23.50	10.50	0.00	17.00	1.16	1.00	73.50	4.41	0.10	44.10	20.00
3.00	30.00	3.00	23.50	10.50	0.00	17.00	1.16	1.00	74.48	4.47	0.10	44.69	20.50
3.00	35.00	4.00	23.50	10.50	0.00	17.00	1.16	1.00	73.50	4.41	0.10	44.10	21.00
3.00	40.00	4.00	23.50	10.50	0.00	17.00	1.16	1.00	74.48	4.47	0.10	44.69	20.00
3.00	45.00	4.00	23.50	10.50	0.00	17.00	1.16	1.00	74.48	4.47	0.10	44.69	20.00
3.00	50.00	5.00	23.50	10.50	0.00	17.00	1.16	1.00	74.48	4.47	0.10	44.69	20.00
3.00	55.00	5.00	25.00	11.50	0.00	18.25	1.24	1.00	74.48	4.47	0.10	44.69	20.50
							1.16		71.87			43.12	19.92

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.80	0.00	1.00	23.50	9.50	0.00	16.50	1.12	1.00	58.80	3.53	0.10	35.28	19.00
2.90	5.00	1.00	23.50	10.50	0.00	17.00	1.16	1.00	64.68	3.88	0.10	38.81	19.00
3.00	10.00	1.00	23.50	10.50	0.00	17.00	1.16	1.00	73.50	4.41	0.10	44.10	19.00
												39.40	
3.00	15.00	2.00	23.50	10.50	0.00	17.00	1.16	1.00	72.52	4.35	0.10	43.51	19.00
3.00	20.00	2.00	23.50	10.50	0.00	17.00	1.16	1.00	73.50	4.41	0.10	44.10	19.50
												43.81	
3.00	25.00	3.00	23.50	10.50	0.00	17.00	1.16	1.00	73.50	4.41	0.10	44.10	19.50
3.00	30.00	3.00	23.50	10.50	0.00	17.00	1.16	1.00	74.48	4.47	0.10	44.69	19.50
												44.39	
3.00	35.00	4.00	23.50	10.50	0.00	17.00	1.16	1.00	73.50	4.41	0.10	44.10	20.00
3.00	40.00	4.00	23.50	10.50	0.00	17.00	1.16	1.00	74.48	4.47	0.10	44.69	20.00
3.00	45.00	4.00	23.50	10.50	0.00	17.00	1.16	1.00	74.48	4.47	0.10	44.69	20.00
												44.49	
3.00	50.00	5.00	23.50	10.50	0.00	17.00	1.16	1.00	74.48	4.47	0.10	44.69	20.00
3.00	55.00	5.00	25.00	11.50	0.00	18.25	1.24	1.00	74.48	4.47	0.10	44.69	20.00
							1.16		71.87			44.69	19.54

SECOND CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.80			22.00	9.00	0.00	15.50	1.05	1.00	43.85	2.63	0.10	26.31	20.00

22-Mar-15	
Experiment 4	
Start UF Sample No	1028730

	REQUIRED	EXPERIMENTAL	VARIANCE
Protein concentration (g/L)	100.00	8.87	-1.13
Ionic strength (M)	0.75	0.75	0.00
Temperature (°C)	20.00	20.5	0.50
Ethanol Concentration (%)	7.75	5.88	-1.87
TMP (Bar)	1.00	1.11	0.11

CLEAN WATER FLUX

Hz	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	PERMEATE FLUX 20°c	TEMPERATURE	NWP (LMH/PSI)	NWP20°c (LMH/PSI)
4.10	5.00	0.00	0.00	2.50	0.17	1.00	105.00	6.30	0.10	63.00	47.88	32.00	12.60	9.58
5.00	10.00	0.00	0.00	5.00	0.34	1.00	170.00	10.20	0.10	102.00	77.52	32.00	10.20	7.75
5.60	15.00	0.00	0.00	7.50	0.51	1.00	250.00	15.00	0.10	150.00	114.00	32.00	10.00	7.60
6.00	20.00	0.00	0.00	10.00	0.68	1.00	280.00	16.80	0.10	168.00	127.68	32.00	8.40	6.38

FIRST CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.40	0.00		24.50	10.00	0.00	17.25	1.17	1.00	64.99	3.90	0.10	38.99	18.50
									64.99				

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.40	0.00	1.00	22.50	10.00	0.00	16.25	1.11	1.00	66.64	4.00	0.10	39.98	20.00
3.40	5.00	1.00	22.50	10.00	0.00	16.25	1.11	1.00	68.60	4.12	0.10	41.16	20.00
3.40	10.00	2.00	22.50	10.00	0.00	16.25	1.11	1.00	70.56	4.23	0.10	42.34	19.50
3.40	15.00	2.00	22.50	10.00	0.00	16.25	1.11	1.00	70.56	4.23	0.10	42.34	19.50
3.40	20.00	2.00	22.50	10.00	0.00	16.25	1.11	1.00	70.56	4.23	0.10	42.34	20.00
3.40	25.00	3.00	22.50	10.00	0.00	16.25	1.11	1.00	71.54	4.29	0.10	42.92	20.50
3.40	30.00	3.00	22.50	10.00	0.00	16.25	1.11	1.00	72.52	4.35	0.10	43.51	20.00
3.40	35.00	4.00	22.50	10.00	0.00	16.25	1.11	1.00	72.52	4.35	0.10	43.51	20.00
3.40	40.00	4.00	22.50	10.00	0.00	16.25	1.11	1.00	72.52	4.35	0.10	43.51	20.00
3.40	45.00	5.00	22.50	10.00	0.00	16.25	1.11	1.00	72.52	4.35	0.10	43.51	19.00
3.40	50.00	5.00	22.50	10.00	0.00	16.25	1.11	1.00	72.52	4.35	0.10	43.51	19.00
3.40	55.00	5.00	22.50	10.00	0.00	16.25	1.11	1.00	72.52	4.35	0.10	43.51	19.00
							1.11		71.13			42.68	19.71

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.40	0.00	1.00	22.50	10.00	0.00	16.25	1.11	1.00	66.64	4.00	0.10	39.98	20.00
3.40	5.00	1.00	22.50	10.00	0.00	16.25	1.11	1.00	68.60	4.12	0.10	41.16	20.00
												40.57	
3.40	10.00	2.00	22.50	10.00	0.00	16.25	1.11	1.00	70.56	4.23	0.10	42.34	19.50
3.40	15.00	2.00	22.50	10.00	0.00	16.25	1.11	1.00	70.56	4.23	0.10	42.34	19.50
3.40	20.00	2.00	22.50	10.00	0.00	16.25	1.11	1.00	70.56	4.23	0.10	42.34	20.00
												42.34	
3.40	25.00	3.00	22.50	10.00	0.00	16.25	1.11	1.00	71.54	4.29	0.10	42.92	20.50
3.40	30.00	3.00	22.50	10.00	0.00	16.25	1.11	1.00	72.52	4.35	0.10	43.51	20.00
												43.22	
3.40	35.00	4.00	22.50	10.00	0.00	16.25	1.11	1.00	72.52	4.35	0.10	43.51	20.00
3.40	40.00	4.00	22.50	10.00	0.00	16.25	1.11	1.00	72.52	4.35	0.10	43.51	20.00
												43.51	
3.40	45.00	5.00	22.50	10.00	0.00	16.25	1.11	1.00	72.52	4.35	0.10	43.51	20.00
3.40	50.00	5.00	22.50	10.00	0.00	16.25	1.11	1.00	72.52	4.35	0.10	43.51	20.00
3.40	55.00	5.00	22.50	10.00	0.00	16.25	1.11	1.00	72.52	4.35	0.10	43.51	20.00
							1.11		71.13			43.51	19.96

SECOND CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.20			22.50	10.00	0.00	16.25	1.11	1.00	61.40	3.68	0.10	36.84	10.00

31-Mar-15	
Experiment 5	
Start UF Sample No	1028731

	REQUIRED	EXPERIMENTAL	VARIANCE
Protein concentration (g/L)	100.00	9.3	-0.7
Ionic strength (M)	2.25	2.25	0.00
Temperature (°C)	10.00	11.5	1.50
Ethanol Concentration (%)	3.25	2.88	-0.37
TMP (Bar)	1.00	1.17	0.17

CLEAN WATER FLUX

Hz	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	PERMEATE FLUX 20°c	TEMPERATURE	NWP (LMH/PSI)	NWP20°c (LMH/PSI)
4.10	5.00	0.00	0.00	2.50	0.17	1.00	110.00	6.60	0.10	66.00	54.12	28.50	13.20	10.82
4.90	10.00	0.00	0.00	5.00	0.34	1.00	160.00	9.60	0.10	96.00	78.72	28.50	9.60	7.87
5.70	15.00	0.00	0.00	7.50	0.51	1.00	205.00	12.30	0.10	123.00	100.86	28.50	8.20	6.72
6.70	20.00	0.00	0.00	10.00	0.68	1.00	250.00	15.00	0.10	150.00	123.00	28.50	7.50	6.15

FIRST CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE	
3.30	0.00		25.00	10.50	0.00	17.75	1.21	1.00	67.93	4.08	0.10	40.76	10.50	
									67.93					

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.20	0.00	1.00	23.00	10.00	0.00	16.50	1.12	1.00	60.76	3.65	0.10	36.46	9.00
3.20	5.00	1.00	23.00	10.00	0.00	16.50	1.12	1.00	60.76	3.65	0.10	36.46	9.50
3.20	10.00	1.00	24.00	10.50	0.00	17.25	1.17	1.00	60.76	3.65	0.10	36.46	10.50
3.10	20.00	2.00	23.00	10.00	0.00	16.50	1.12	1.00	58.31	3.50	0.10	34.99	11.00
3.10	25.00	2.00	24.00	10.50	0.00	17.25	1.17	1.00	58.80	3.53	0.10	35.28	11.50
3.10	30.00	3.00	24.00	10.50	0.00	17.25	1.17	1.00	58.31	3.50	0.10	34.99	11.50
3.10	35.00	3.00	24.00	10.50	0.00	17.25	1.17	1.00	58.31	3.50	0.10	34.99	11.50
3.10	40.00	3.00	24.00	10.50	0.00	17.25	1.17	1.00	58.80	3.53	0.10	35.28	11.00
3.10	45.00	4.00	24.00	10.50	0.00	17.25	1.17	1.00	58.80	3.53	0.10	35.28	11.00
3.10	50.00	4.00	24.00	10.50	0.00	17.25	1.17	1.00	58.80	3.53	0.10	35.28	10.50
3.10	55.00	4.00	24.00	10.50	0.00	17.25	1.17	1.00	57.82	3.47	0.10	34.69	11.00
3.10	60.00	5.00	24.00	10.50	0.00	17.25	1.17	1.00	57.82	3.47	0.10	34.69	10.50
3.10	65.00	5.00	24.00	10.50	0.00	17.25	1.17	1.00	57.82	3.47	0.10	34.69	11.00
							1.16		58.91			35.35	10.73

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.20	0.00	1.00	23.00	10.00	0.00	16.50	1.12	1.00	60.76	3.65	0.10	36.46	9.00
3.20	5.00	1.00	23.00	10.00	0.00	16.50	1.12	1.00	60.76	3.65	0.10	36.46	9.50
3.20	10.00	1.00	24.00	10.50	0.00	17.25	1.17	1.00	60.76	3.65	0.10	36.46	10.50
												36.46	
3.10	20.00	2.00	24.00	10.50	0.00	17.25	1.17	1.00	58.31	3.50	0.10	34.99	11.00
3.10	25.00	2.00	24.00	10.50	0.00	17.25	1.17	1.00	58.80	3.53	0.10	35.28	11.50
												35.13	
3.10	30.00	3.00	24.00	10.50	0.00	17.25	1.17	1.00	58.31	3.50	0.10	34.99	11.50
3.10	35.00	3.00	24.00	10.50	0.00	17.25	1.17	1.00	58.31	3.50	0.10	34.99	11.50
3.10	40.00	3.00	24.00	10.50	0.00	17.25	1.17	1.00	58.80	3.53	0.10	35.28	11.00
												35.08	
3.10	45.00	4.00	24.00	10.50	0.00	17.25	1.17	1.00	58.80	3.53	0.10	35.28	11.00
3.10	50.00	4.00	24.00	10.50	0.00	17.25	1.17	1.00	58.80	3.53	0.10	35.28	10.50
3.10	55.00	4.00	24.00	10.50	0.00	17.25	1.17	1.00	57.82	3.47	0.10	34.69	11.00
												35.08	
3.10	60.00	5.00	24.00	10.50	0.00	17.25	1.17	1.00	57.82	3.47	0.10	34.69	10.50
3.10	65.00	5.00	24.00	10.50	0.00	17.25	1.17	1.00	57.82	3.47	0.10	34.69	11.00
							1.17		58.91			34.69	10.73

SECOND CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.10			24.00	10.00	0.00	17.00	1.16	1.00	43.85	2.63	0.10	26.31	10.00

16-Mar-15	
Experiment 6	
Start UF Sample No	1028732

	REQUIRED	EXPERIMENTAL	VARIANCE
Protein concentration (g/L)	100.00	9.19	-0.81
Ionic strength (M)	2.25	2.25	0.00
Temperature (°C)	10.00	12	2.00
Ethanol Concentration (%)	7.75	6.69	-1.06
TMP (Bar)	1.00	1.14	0.14

CLEAN WATER FLUX

Hz	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	PERMEATE FLUX 20°c	TEMPERATURE	NWP (LMH/PSI)	NWP20°c (LMH/PSI)
4.10	5.00	0.00	0.00	2.50	0.17	1.00	100.00	6.00	0.10	60.00	52.20	26.00	12.00	10.44
5.00	10.00	0.00	0.00	5.00	0.34	1.00	140.00	8.40	0.10	84.00	73.08	26.00	8.40	7.31
5.60	15.00	0.00	0.00	7.50	0.51	1.00	185.00	11.10	0.10	111.00	96.57	26.00	7.40	6.44
6.20	20.00	0.00	0.00	10.00	0.68	1.00	220.00	13.20	0.10	132.00	114.84	26.00	6.60	5.74

FIRST CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.30	0.00		22.50	9.50	0.00	16.00	1.09	1.00	53.40	3.20	0.10	32.04	10.00
									53.40				

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.30	0.00	1.00	23.50	10.00	0.00	16.75	1.14	1.00	56.84	3.41	0.10	34.10	12.00
3.30	5.00	1.00	23.50	10.00	0.00	16.75	1.14	1.00	56.84	3.41	0.10	34.10	12.00
3.30	10.00	2.00	23.50	10.00	0.00	16.75	1.14	1.00	58.80	3.53	0.10	35.28	12.00
3.30	15.00	2.00	23.50	10.00	0.00	16.75	1.14	1.00	58.80	3.53	0.10	35.28	11.50
3.30	20.00	2.00	23.50	10.00	0.00	16.75	1.14	1.00	58.80	3.53	0.10	35.28	11.50
3.30	25.00	3.00	23.50	10.00	0.00	16.75	1.14	1.00	59.78	3.59	0.10	35.87	11.00
3.30	30.00	3.00	23.50	10.00	0.00	16.75	1.14	1.00	59.78	3.59	0.10	35.87	11.00
3.30	35.00	3.00	23.50	10.00	0.00	16.75	1.14	1.00	60.76	3.65	0.10	36.46	11.00
3.30	40.00	4.00	23.50	10.00	0.00	16.75	1.14	1.00	60.76	3.65	0.10	36.46	10.50
3.30	45.00	4.00	23.50	10.00	0.00	16.75	1.14	1.00	59.78	3.59	0.10	35.87	10.50
3.30	50.00	4.00	23.50	10.00	0.00	16.75	1.14	1.00	60.76	3.65	0.10	36.46	10.50
3.30	55.00	5.00	23.50	10.00	0.00	16.75	1.14	1.00	60.76	3.65	0.10	36.46	10.00
3.30	60.00	5.00	23.50	10.00	0.00	16.75	1.14	1.00	60.76	3.65	0.10	36.46	10.00
							1.14		59.48			35.69	11.04

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.30	0.00	1.00	23.50	10.00	0.00	16.75	1.14	1.00	56.84	3.41	0.10	34.10	12.00
3.30	5.00	1.00	23.50	10.00	0.00	16.75	1.14	1.00	56.84	3.41	0.10	34.10	12.00
												34.10	
3.30	10.00	2.00	23.50	10.00	0.00	16.75	1.14	1.00	58.80	3.53	0.10	35.28	12.00
3.30	15.00	2.00	23.50	10.00	0.00	16.75	1.14	1.00	58.80	3.53	0.10	35.28	11.50
3.30	20.00	2.00	23.50	10.00	0.00	16.75	1.14	1.00	58.80	3.53	0.10	35.28	11.50
												35.28	
3.30	25.00	3.00	23.50	10.00	0.00	16.75	1.14	1.00	59.78	3.59	0.10	35.87	11.00
3.30	30.00	3.00	23.50	10.00	0.00	16.75	1.14	1.00	59.78	3.59	0.10	35.87	11.00
3.30	35.00	3.00	23.50	10.00	0.00	16.75	1.14	1.00	60.76	3.65	0.10	36.46	11.00
												36.06	
3.30	40.00	4.00	23.50	10.00	0.00	16.75	1.14	1.00	60.76	3.65	0.10	36.46	10.50
3.30	45.00	4.00	23.50	10.00	0.00	16.75	1.14	1.00	59.78	3.59	0.10	35.87	10.50
3.30	50.00	4.00	23.50	10.00	0.00	16.75	1.14	1.00	60.76	3.65	0.10	36.46	10.50
												36.26	
3.30	55.00	5.00	23.50	10.00	0.00	16.75	1.14	1.00	60.76	3.65	0.10	36.46	10.00
3.30	60.00	5.00	23.50	10.00	0.00	16.75	1.14	1.00	60.76	3.65	0.10	36.46	10.00
							1.14		59.48			36.46	11.04

SECOND CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.30			23.50	10.00	0.00	16.75	1.14	1.00	58.06	3.48	0.10	34.84	10.00

08-Mar-15	
Experiment 7	
Start UF Sample No	1028733

	REQUIRED	EXPERIMEN	VARIANCE
Protein concentration (g/L)	100.00	8.65	-1.35
Ionic strength (M)	2.25	2.25	0.00
Temperature (°C)	20.00	19.5	-0.50
Ethanol Concentration (%)	3.25	2.39	-0.86
TMP (Bar)	1.00	1.19	0.19

CLEAN WATER FLUX

Hz	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	PERMEATE FLUX 20°C	EMPERATUR	NWP (LMH/PSI)	NWP20°c (LMH/PSI)
4.20	5.00	0.00	0.00	2.50	0.17	1.00	135.00	8.10	0.10	81.00	65.21	29.50	16.20	13.04
4.90	10.00	0.00	0.00	5.00	0.34	1.00	195.00	11.70	0.10	117.00	94.19	29.50	11.70	9.42
5.40	15.00	0.00	0.00	7.50	0.51	1.00	235.00	14.10	0.10	141.00	113.51	29.50	9.40	7.57
5.90	20.00	0.00	0.00	10.00	0.68	1.00	280.00	16.80	0.10	168.00	135.24	29.50	8.40	6.76

FIRST CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.80	0.00		22.50	9.00	0.00	15.75	1.07	1.00	81.25	4.88	0.10	48.75	10.00
									81.25			48.75	

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.60	0.00	1.00	24.00	9.50	0.00	16.75	1.14	1.00	79.38	4.76	0.10	47.63	19.50
3.50	5.00	1.00	24.50	9.50	0.00	17.00	1.16	1.00	77.42	4.65	0.10	46.45	19.50
3.40	10.00	2.00	24.50	9.50	0.00	17.00	1.16	1.00	74.48	4.47	0.10	44.69	19.50
3.40	15.00	2.00	24.50	9.50	0.00	17.00	1.16	1.00	74.48	4.47	0.10	44.69	19.00
3.40	20.00	2.00	24.50	9.50	0.00	17.00	1.16	1.00	74.48	4.47	0.10	44.69	19.00
3.40	25.00	3.00	25.00	10.00	0.00	17.50	1.19	1.00	74.48	4.47	0.10	44.69	19.00
3.40	30.00	3.00	25.50	10.00	0.00	17.75	1.21	1.00	74.48	4.47	0.10	44.69	19.00
3.40	35.00	4.00	26.50	10.00	0.00	18.25	1.24	1.00	74.48	4.47	0.10	44.69	19.00
3.40	40.00	4.00	26.50	10.00	0.00	18.25	1.24	1.00	74.48	4.47	0.10	44.69	19.00
3.40	45.00	5.00	26.50	10.00	0.00	18.25	1.24	1.00	74.48	4.47	0.10	44.69	19.50
3.40	50.00	5.00	26.50	10.00	0.00	18.25	1.24	1.00	74.48	4.47	0.10	44.69	19.50
							1.19		75.19			45.12	19.23

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.60	0.00	1.00	24.00	9.50	0.00	16.75	1.14	1.00	79.38	4.76	0.10	47.63	19.50
3.50	5.00	1.00	24.50	9.50	0.00	17.00	1.16	1.00	77.42	4.65	0.10	46.45	19.50
												47.04	
3.40	10.00	2.00	24.50	9.50	0.00	17.00	1.16	1.00	74.48	4.47	0.10	44.69	19.50
3.40	15.00	2.00	24.50	9.50	0.00	17.00	1.16	1.00	74.48	4.47	0.10	44.69	19.00
3.40	20.00	2.00	24.50	9.50	0.00	17.00	1.16	1.00	74.48	4.47	0.10	44.69	19.00
												44.69	
3.40	25.00	3.00	25.00	10.00	0.00	17.50	1.19	1.00	74.48	4.47	0.10	44.69	19.00
3.40	30.00	3.00	25.50	10.00	0.00	17.75	1.21	1.00	74.48	4.47	0.10	44.69	19.00
												44.69	
3.40	35.00	4.00	26.50	10.00	0.00	18.25	1.24	1.00	74.48	4.47	0.10	44.69	19.00
3.40	40.00	4.00	26.50	10.00	0.00	18.25	1.24	1.00	74.48	4.47	0.10	44.69	19.00
												44.69	
3.40	45.00	5.00	26.50	10.00	0.00	18.25	1.24	1.00	74.48	4.47	0.10	44.69	19.50
3.40	50.00	5.00	26.50	10.00	0.00	18.25	1.24	1.00	74.48	4.47	0.10	44.69	19.50
									75.19			44.69	19.23

SECOND CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.30			22.00	10.00	0.00	16.00	1.09	1.00	61.47	3.69	0.10	36.88	19.50

20-Mar-15	
Experiment 8	
Start UF Sample No	1028734

	REQUIRED	EXPERIMENTAL	VARIANCE
Protein concentration (g/L)	100.00	8.75	-1.25
Ionic strength (M)	2.25	2.25	0.00
Temperature (°C)	20.00	21	1.00
Ethanol Concentration (%)	7.75	5.99	-1.76
TMP (Bar)	1.00	1.12	0.12

CLEAN WATER FLUX

Hz	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	PERMEATE FLUX 20°c	TEMPERATURE	NWP (LMH/PSI)	NWP20°c (LMH/PSI)
3.90	5.00	0.00	0.00	2.50	0.17	1.00	92.00	5.52	0.10	55.20	46.92	27.00	11.04	9.38
4.70	10.00	0.00	0.00	5.00	0.34	1.00	130.00	7.80	0.10	78.00	66.30	27.00	7.80	6.63
5.30	15.00	0.00	0.00	7.50	0.51	1.00	170.00	10.20	0.10	102.00	86.70	27.00	6.80	5.78
5.90	20.00	0.00	0.00	10.00	0.68	1.00	210.00	12.60	0.10	126.00	107.10	27.00	6.30	5.36

FIRST CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.40	0.00		22.50	10.00	0.00	16.25	1.11	1.00	57.93	3.48	0.10	34.76	15.00
									57.93				

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.40	0.00	1.00	22.50	10.00	0.00	16.25	1.11	1.00	64.68	3.88	0.10	38.81	20.00
3.40	5.00	1.00	22.50	10.00	0.00	16.25	1.11	1.00	64.68	3.88	0.10	38.81	20.00
3.40	10.00	1.00	22.50	10.00	0.00	16.25	1.11	1.00	65.66	3.94	0.10	39.40	20.00
3.40	15.00	2.00	22.50	10.00	0.00	16.25	1.11	1.00	66.64	4.00	0.10	39.98	21.00
3.40	20.00	2.00	23.00	10.50	0.00	16.75	1.14	1.00	68.60	4.12	0.10	41.16	20.00
3.40	25.00	3.00	21.00	9.50	0.00	15.25	1.04	1.00	62.72	3.76	0.10	37.63	20.00
3.50	30.00	3.00	22.50	10.50	0.00	16.50	1.12	1.00	68.60	4.12	0.10	41.16	20.00
3.50	40.00	4.00	23.50	11.00	0.00	17.25	1.17	1.00	69.58	4.17	0.10	41.75	20.00
3.50	45.00	4.00	24.00	11.00	0.00	17.50	1.19	1.00	69.58	4.17	0.10	41.75	20.00
3.40	50.00	5.00	22.50	10.00	0.00	16.25	1.11	1.00	64.68	3.88	0.10	38.81	20.00
3.40	55.00	5.00	23.00	11.00	0.00	17.00	1.16	1.00	64.68	3.88	0.10	38.81	20.00
							1.12		66.37			39.82	20.09

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.40	0.00	1.00	22.50	10.00	0.00	16.25	1.11	1.00	64.68	3.88	0.10	38.81	20.00
3.40	5.00	1.00	22.50	10.00	0.00	16.25	1.11	1.00	64.68	3.88	0.10	38.81	20.00
3.40	10.00	1.00	22.50	10.00	0.00	16.25	1.11	1.00	65.66	3.94	0.10	39.40	20.00
												39.00	
3.40	15.00	2.00	22.50	10.00	0.00	16.25	1.11	1.00	66.64	4.00	0.10	39.98	21.00
3.40	20.00	2.00	23.00	10.50	0.00	16.75	1.14	1.00	68.60	4.12	0.10	41.16	20.00
												40.57	
3.40	25.00	3.00	21.00	9.50	0.00	15.25	1.04	1.00	62.72	3.76	0.10	37.63	20.00
3.50	30.00	3.00	22.50	10.50	0.00	16.50	1.12	1.00	68.60	4.12	0.10	41.16	20.00
												39.40	
3.50	40.00	4.00	23.50	11.00	0.00	17.25	1.17	1.00	69.58	4.17	0.10	41.75	20.00
3.50	45.00	4.00	24.00	11.00	0.00	17.50	1.19	1.00	69.58	4.17	0.10	41.75	20.00
												41.75	
3.40	50.00	5.00	22.50	10.00	0.00	16.25	1.11	1.00	64.68	3.88	0.10	38.81	20.00
3.40	55.00	5.00	23.00	11.00	0.00	17.00	1.16	1.00	64.68	3.88	0.10	38.81	20.00
							1.12		66.37			38.81	20.09

SECOND CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.80			20.05	9.50	0.00	14.78	1.01	1.00	60.01	3.60	0.10	36.01	21.00

01-Apr-15	
Experiment 9	
Start UF Sample No	1028735

	REQUIRED	EXPERIMENTAL	VARIANCE
Protein concentration (g/L)	180.00	16.03	-1.97
Ionic strength (M)	0.75	0.75	0.00
Temperature (°C)	10.00	11	1.00
Ethanol Concentration (%)	3.25		-3.25
TMP (Bar)	1.00	0.97	-0.03

CLEAN WATER FLUX

Hz	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	PERMEATE FLUX 20°c	TEMPERATURE	NWP (LMH/PSI)	NWP20°c (LMH/PSI)
4.10	5.00	0.00	0.00	2.50	0.17	1.00	108.00	6.48	0.10	64.80	59.62	23.50	12.96	11.92
4.80	10.00	0.00	0.00	5.00	0.34	1.00	159.00	9.54	0.10	95.40	87.77	23.50	9.54	8.78
5.70	15.00	0.00	0.00	7.50	0.51	1.00	199.00	11.94	0.10	119.40	109.85	23.50	7.96	7.32
6.60	20.00	0.00	0.00	10.00	0.68	1.00	240.00	14.40	0.10	144.00	132.48	23.50	7.20	6.62

FIRST CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.30	0.00		37.50	10.00	0.00	23.75	1.62	1.00	44.36	2.66	0.10	26.62	10.00
									44.36				

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.30	0.00	1.00	19.50	9.00	0.00	14.25	0.97	1.00	32.34	1.94	0.10	19.40	11.00
2.30	10.00	1.00	19.50	9.00	0.00	14.25	0.97	1.00	33.32	2.00	0.10	19.99	11.00
2.30	20.00	2.00	19.50	9.00	0.00	14.25	0.97	1.00	34.30	2.06	0.10	20.58	10.50
2.30	30.00	2.00	19.50	9.00	0.00	14.25	0.97	1.00	34.30	2.06	0.10	20.58	11.00
2.30	40.00	3.00	19.50	9.00	0.00	14.25	0.97	1.00	34.30	2.06	0.10	20.58	11.00
2.30	55.00	4.00	19.50	9.00	0.00	14.25	0.97	1.00	35.28	2.12	0.10	21.17	11.00
2.30	70.00	5.00	19.50	9.00	0.00	14.25	0.97	1.00	35.28	2.12	0.10	21.17	11.00
2.30	75.00	5.00	19.50	9.00	0.00	14.25	0.97	1.00	35.28	2.12	0.10	21.17	11.00
							0.97		34.30			20.58	10.94

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.30		1.00	19.50	9.00	0.00	14.25	0.97	1.00	28.99	1.74	0.10	17.39	16.00
2.30		2.00	19.50	9.00	0.00	14.25	0.97	1.00	30.74	1.84	0.10	18.44	15.00
2.30		3.00	19.50	9.00	0.00	14.25	0.97	1.00	31.02	1.86	0.10	18.61	15.00
2.30		4.00	19.50	9.00	0.00	14.25	0.97	1.00	31.41	1.88	0.10	18.85	15.00
2.30		5.00	19.50	9.00	0.00	14.25	0.97	1.00	31.71	1.90	0.10	19.03	15.50
												18.46	

SECOND CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.30			19.50	9.00	0.00	14.25	0.97	1.00	30.00	1.80	0.10	18.00	11.00

12-Mar-15	
Experiment 10	
Start UF Sample No	1028736

	REQUIRED	EXPERIMENTAL	VARIANCE
Protein concentration (g/L)	180.00	16.37	-1.63
Ionic strength (M)	0.75	1.5	-0.75
Temperature (°C)	10.00	12	2.00
Ethanol Concentration (%)	7.75	5.65	-2.10
TMP (Bar)	1.00	1.65	0.65

CLEAN WATER FLUX

Hz	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	PERMEATE FLUX 20°c	TEMPERATURE	NWP (LMH/PSI)	NWP20°c (LMH/PSI)
4.40	5.00	0.00	0.00	2.50	0.17	1.00	94.00	5.64	0.10	56.40	51.89	23.50	11.28	10.38
5.20	10.00	0.00	0.00	5.00	0.34	1.00	152.00	9.12	0.10	91.20	83.90	23.50	9.12	8.39
5.60	15.00	0.00	0.00	7.50	0.51	1.00	205.00	12.30	0.10	123.00	113.16	23.50	8.20	7.54
6.10	20.00	0.00	0.00	10.00	0.68	1.00	222.00	13.32	0.10	133.20	122.54	23.50	6.66	6.13

FIRST CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.50	0.00		35.00	13.00	0.00	24.00	1.63	1.00	31.44	1.89	0.10	18.86	10.00
									31.44				

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.40	0.00	1.00	19.50	8.00	0.00	13.75	0.94	1.00	29.40	1.76	0.10	17.64	12.00
2.40	10.00	1.00	18.50	8.00	0.00	13.25	0.90	1.00	30.38	1.82	0.10	18.23	12.00
2.40	20.00	1.00	18.00	8.00	0.00	13.00	0.88	1.00	31.36	1.88	0.10	18.82	12.00
2.50	30.00	2.00	20.50	8.50	0.00	14.50	0.99	1.00	33.32	2.00	0.10	19.99	12.00
2.50	40.00	2.00	20.50	8.50	0.00	14.50	0.99	1.00	33.32	2.00	0.10	19.99	12.00
2.50	50.00	2.00	20.50	8.50	0.00	14.50	0.99	1.00	33.32	2.00	0.10	19.99	12.00
2.50	60.00	3.00	20.00	8.50	0.00	14.25	0.97	1.00	33.32	2.00	0.10	19.99	12.00
2.50	70.00	3.00	20.00	8.50	0.00	14.25	0.97	1.00	32.34	1.94	0.10	19.40	11.00
2.50	80.00	3.00	20.00	8.50	0.00	14.25	0.97	1.00	33.32	2.00	0.10	19.99	11.00
2.50	90.00	4.00	20.00	8.50	0.00	14.25	0.97	1.00	34.30	2.06	0.10	20.58	11.00
2.50	100.00	4.00	20.00	8.50	0.00	14.25	0.97	1.00	34.30	2.06	0.10	20.58	10.50
2.50	115.00	5.00	20.00	9.00	0.00	14.50	0.99	1.00	33.32	2.00	0.10	19.99	10.00
2.50	125.00	5.00	20.00	9.00	0.00	14.50	0.99	1.00	34.30	2.06	0.10	20.58	10.00
							0.96		32.79			19.68	11.35

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.60		1.00	20.50	8.50	0.00	14.50	0.99	1.00	25.43	1.53	0.10	15.26	12.00
2.60		2.00	20.50	8.50	0.00	14.50	0.99	1.00	28.96	1.74	0.10	17.38	12.00
2.60		3.00	20.00	8.50	0.00	14.25	0.97	1.00	29.73	1.78	0.10	17.84	11.00
2.60		4.00	20.00	8.50	0.00	14.25	0.97	1.00	30.19	1.81	0.10	18.11	10.50
2.60		5.00	20.00	8.50	0.00	14.25	0.97	1.00	30.33	1.82	0.10	18.20	10.00
												17.36	

SECOND CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.50			19.20	12.00	0.00	15.60	1.06	1.00	28.19	1.69	0.10	16.91	10.00

10-Mar-15	
Experiment 11	
Start UF Sample No	1028737

	REQUIRED	EXPERIMENTAL	VARIANCE
Protein concentration (g/L)	180.00	15.96	-2.04
Ionic strength (M)	0.75	1.5	-0.75
Temperature (°C)	20.00	20.5	0.50
Ethanol Concentration (%)	3.25	2.63	-0.62
TMP (Bar)	1.00	1.67	0.67

CLEAN WATER FLUX

Hz	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	PERMEATE FLUX 20°c	TEMPERATURE	NWP (LMH/PSI)	NWP20°c (LMH/PSI)
4.20	5.00	0.00	0.00	2.50	0.17	1.00	92.00	5.52	0.10	55.20	48.02	26.00	11.04	9.60
4.90	10.00	0.00	0.00	5.00	0.34	1.00	146.00	8.76	0.10	87.60	76.21	26.00	8.76	7.62
5.30	15.00	0.00	0.00	7.50	0.51	1.00	119.00	7.14	0.10	71.40	62.12	26.00	4.76	4.14
5.90	20.00	0.00	0.00	10.00	0.68	1.00	217.00	13.02	0.10	130.20	113.27	26.00	6.51	5.66

FIRST CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.50	0.00		35.00	15.00	0.00	25.00	1.70	1.00	61.35	3.68	0.10	36.81	18.00
									61.35				

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.60	0.00	1.00	15.00	13.00	0.00	14.00	0.95	1.00	41.16	2.47	0.10	24.70	18.00
2.60	5.00	1.00	15.00	13.00	0.00	14.00	0.95	1.00	43.12	2.59	0.10	25.87	18.00
2.60	10.00	1.00	15.00	13.00	0.00	14.00	0.95	1.00	43.12	2.59	0.10	25.87	18.50
2.60	15.00	1.00	15.00	13.00	0.00	14.00	0.95	1.00	44.10	2.65	0.10	26.46	18.50
2.60	25.00	2.00	15.00	13.00	0.00	14.00	0.95	1.00	44.10	2.65	0.10	26.46	19.00
2.60	30.00	2.00	15.00	13.00	0.00	14.00	0.95	1.00	45.08	2.70	0.10	27.05	19.50
2.60	35.00	2.00	15.00	13.00	0.00	14.00	0.95	1.00	45.08	2.70	0.10	27.05	19.50
2.60	45.00	3.00	15.50	13.50	0.00	14.50	0.99	1.00	46.06	2.76	0.10	27.64	20.00
2.60	50.00	3.00	15.50	13.50	0.00	14.50	0.99	1.00	46.06	2.76	0.10	27.64	20.00
2.60	60.00	4.00	16.00	13.50	0.00	14.75	1.00	1.00	46.06	2.76	0.10	27.64	20.00
2.60	65.00	4.00	16.50	14.00	0.00	15.25	1.04	1.00	46.06	2.76	0.10	27.64	20.00
2.60	70.00	4.00	16.50	14.00	0.00	15.25	1.04	1.00	46.06	2.76	0.10	27.64	20.50
2.60	80.00	5.00	16.50	14.00	0.00	15.25	1.04	1.00	47.04	2.82	0.10	28.22	20.50
2.60	85.00	5.00	16.50	14.00	0.00	15.25	1.04	1.00	47.04	2.82	0.10	28.22	20.00
2.60	90.00	5.00	16.00	13.50	0.00	14.75	1.00	1.00	47.04	2.82	0.10	28.22	20.00
							0.99		45.15			27.09	19.47

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.60		1.00	35.00	13.00	0.00	24.00	1.63	1.00	38.28	2.30	0.10	22.97	19.00
2.60		2.00	35.00	13.00	0.00	24.00	1.63	1.00	41.38	2.48	0.10	24.83	19.50
2.60		3.00	35.00	13.00	0.00	24.00	1.63	1.00	42.74	2.56	0.10	25.64	20.00
2.60		4.00	35.00	13.00	0.00	24.00	1.63	1.00	43.28	2.60	0.10	25.97	20.00
2.60		5.00	35.00	13.00	0.00	24.00	1.63	1.00	43.96	2.64	0.10	26.38	20.00

SECOND CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.60			23.00	10.00	0.00	16.50	1.12	1.00	41.46	2.49	0.10	24.88	20.00

30-Mar-15	
Experiment 12	
Start UF Sample No	1028738

	REQUIRED	EXPERIMENTAL	VARIANCE
Protein concentration (g/L)	180.00	16.16	-1.84
Ionic strength (M)	0.75	0.75	0.00
Temperature (°C)	20.00	20	0.00
Ethanol Concentration (%)	7.75	5.22	-2.53
TMP (Bar)	1.00	1.67	0.67

CLEAN WATER FLUX

Hz	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	PERMEATE FLUX 20°c	TEMPERATURE	NWP (LMH/PSI)	NWP20°c (LMH/PSI)
3.20	5.00	0.00	0.00	2.50	0.17	1.00	60.00	3.60	0.10	36.00	29.52	24.00	7.20	5.90
4.10	10.00	0.00	0.00	5.00	0.34	1.00	96.00	5.76	0.10	57.60	47.23	24.00	5.76	4.72
4.80	15.00	0.00	0.00	7.50	0.51	1.00	141.00	8.46	0.10	84.60	69.37	24.00	5.64	4.62
5.30	20.00	0.00	0.00	10.00	0.68	1.00	170.00	10.20	0.10	102.00	83.64	24.00	5.10	4.18

FIRST CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.00	0.00		35.00	15.00	0.00	25.00	1.70	1.00	55.40	3.32	0.10	33.24	18.00
									55.40				

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.60	0.00	1.00	15.00	15.00	0.00	15.00	1.02	1.00	43.12	2.59	0.10	25.87	20.00
2.60	10.00	1.00	15.00	15.00	0.00	15.00	1.02	1.00	43.12	2.59	0.10	25.87	20.00
2.60	15.00	2.00	14.50	15.00	0.00	14.75	1.00	1.00	44.10	2.65	0.10	26.46	20.00
2.60	20.00	2.00	14.50	15.00	0.00	14.75	1.00	1.00	45.08	2.70	0.10	27.05	20.00
2.60	30.00	3.00	14.50	15.00	0.00	14.75	1.00	1.00	45.08	2.70	0.10	27.05	20.00
2.60	40.00	4.00	14.50	15.00	0.00	14.75	1.00	1.00	47.04	2.82	0.10	28.22	20.00
2.60	45.00	4.00	14.50	15.00	0.00	14.75	1.00	1.00	47.04	2.82	0.10	28.22	20.00
2.60	53.00	5.00	13.50	14.50	0.00	14.00	0.95	1.00	47.04	2.82	0.10	28.22	20.00
2.60	58.00	5.00	13.50	14.50	0.00	14.00	0.95	1.00	47.04	2.82	0.10	28.22	20.00
							1.00		45.41			27.24	20.00

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.60		1.00	35.00	15.00	0.00	25.00	1.70	1.00	41.72	2.50	0.10	25.03	20.00
2.60		2.00	34.50	15.00	0.00	24.75	1.68	1.00	43.67	2.62	0.10	26.20	20.00
2.60		3.00	34.50	15.00	0.00	24.75	1.68	1.00	42.61	2.56	0.10	25.57	20.00
2.60		4.00	34.50	15.00	0.00	24.75	1.68	1.00	43.60	2.62	0.10	26.16	20.00
2.60		5.00	33.50	14.50	0.00	24.00	1.63	1.00	44.64	2.68	0.10	26.78	20.00
												25.95	

SECOND CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.60			33.50	14.50	0.00	24.00	1.63	1.00	43.84	2.63	0.10	26.30	20.00

22-Mar-15	
Experiment 13	
Start UF Sample No	1028739

	REQUIRED	EXPERIMENTAL	VARIANCE
Protein concentration (g/L)	180.00	15.4	-2.6
Ionic strength (M)	2.25	2.25	0.00
Temperature (°C)	10.00	12	2.00
Ethanol Concentration (%)	3.25		-3.25
TMP (Bar)	1.00	1.7	0.70

CLEAN WATER FLUX

Hz	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	PERMEATE FLUX 20°c	TEMPERATURE	NWP (LMH/PSI)	NWP20°c (LMH/PSI)
4.00	5.00	0.00	0.00	2.50	0.17	1.00	98.00	5.88	0.10	58.80	50.57	26.50	11.76	10.11
4.80	10.00	0.00	0.00	5.00	0.34	1.00	160.00	9.60	0.10	96.00	82.56	26.50	9.60	8.26
5.50	15.00	0.00	0.00	7.50	0.51	1.00	241.00	14.46	0.10	144.60	124.36	26.50	9.64	8.29
5.90	20.00	0.00	0.00	10.00	0.68	1.00	260.00	15.60	0.10	156.00	134.16	26.50	7.80	6.71

FIRST CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.90	0.00		40.00	10.00	0.00	25.00	1.70	1.00	42.97	2.58	0.10	25.78	10.00
									42.97				

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.40	0.00	1.00	18.00	10.00	0.00	14.00	0.95	1.00	32.34	1.94	0.10	19.40	12.00
2.40	10.00	1.00	19.00	10.00	0.00	14.50	0.99	1.00	32.34	1.94	0.10	19.40	12.00
2.40	25.00	2.00	19.00	10.00	0.00	14.50	0.99	1.00	32.34	1.94	0.10	19.40	12.00
2.40	35.00	2.00	19.50	10.50	0.00	15.00	1.02	1.00	33.32	2.00	0.10	19.99	11.50
2.40	45.00	2.00	19.50	10.50	0.00	15.00	1.02	1.00	33.32	2.00	0.10	19.99	11.00
2.40	55.00	3.00	20.00	10.50	0.00	15.25	1.04	1.00	33.32	2.00	0.10	19.99	11.00
2.40	65.00	3.00	20.00	10.50	0.00	15.25	1.04	1.00	33.32	2.00	0.10	19.99	10.50
2.40	75.00	4.00	20.00	10.50	0.00	15.25	1.04	1.00	32.34	1.94	0.10	19.40	10.00
2.40	85.00	4.00	20.00	10.50	0.00	15.25	1.04	1.00	33.32	2.00	0.10	19.99	10.00
2.40	100.00	5.00	20.00	10.50	0.00	15.25	1.04	1.00	33.32	2.00	0.10	19.99	10.00
2.40	110.00	5.00	20.00	10.50	0.00	15.25	1.04	1.00	33.32	2.00	0.10	19.99	10.00
							1.02		32.96			19.78	10.91

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.40		1.00	38.00	10.00	0.00	24.00	1.63	1.00	29.10	1.75	0.10	17.46	12.00
2.40		2.00	39.00	10.00	0.00	24.50	1.67	1.00	30.47	1.83	0.10	18.28	11.00
2.40		3.00	40.00	10.50	0.00	25.25	1.72	1.00	30.61	1.84	0.10	18.37	10.00
2.40		4.00	40.00	10.50	0.00	25.25	1.72	1.00	30.72	1.84	0.10	18.43	10.00
2.40		5.00	40.00	10.50	0.00	25.25	1.72	1.00	30.97	1.86	0.10	18.58	10.00
												18.22	

SECOND CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.40			40.00	10.50	0.00	25.25	1.72	1.00	32.79	1.97	0.10	19.67	10.00

27-Mar-15	
Experiment 14	
Start UF Sample No	1028740

	REQUIRED	EXPERIMENTAL	VARIANCE
Protein concentration (g/L)	180.00	15.33	-2.67
Ionic strength (M)	2.25	2.25	0.00
Temperature (°C)	10.00	12	2.00
Ethanol Concentration (%)	7.75		-7.75
TMP (Bar)	1.00	1.64	0.64

CLEAN WATER FLUX

Hz	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	PERMEATE FLUX 20°c	TEMPERATURE	NWP (LMH/PSI)	NWP20°c (LMH/PSI)
3.70	5.00	0.00	0.00	2.50	0.17	1.00	65.00	3.90	0.10	39.00	34.32	25.50	7.80	6.86
4.50	10.00	0.00	0.00	5.00	0.34	1.00	99.00	5.94	0.10	59.40	52.27	25.50	5.94	5.23
5.00	15.00	0.00	0.00	7.50	0.51	1.00	139.00	8.34	0.10	83.40	73.39	25.50	5.56	4.89
5.50	20.00	0.00	0.00	10.00	0.68	1.00	179.00	10.74	0.10	107.40	94.51	25.50	5.37	4.73

FIRST CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.10	0.00		40.00	10.00	0.00	25.00	1.70	1.00	35.29	2.12	0.10	21.17	11.00
									35.29				

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.50	0.00	1.00	18.00	10.00	0.00	14.00	0.95	1.00	29.40	1.76	0.10	17.64	12.00
2.50	10.00	1.00	18.00	10.00	0.00	14.00	0.95	1.00	30.38	1.82	0.10	18.23	12.00
2.50	20.00	1.00	18.00	10.00	0.00	14.00	0.95	1.00	31.36	1.88	0.10	18.82	11.50
2.50	30.00	2.00	18.00	10.00	0.00	14.00	0.95	1.00	31.36	1.88	0.10	18.82	11.50
2.50	40.00	2.00	18.00	10.00	0.00	14.00	0.95	1.00	32.34	1.94	0.10	19.40	11.50
2.50	60.00	3.00	18.50	10.00	0.00	14.25	0.97	1.00	32.34	1.94	0.10	19.40	11.00
2.50	70.00	3.00	18.00	10.00	0.00	14.00	0.95	1.00	32.34	1.94	0.10	19.40	11.00
2.50	90.00	4.00	18.50	10.00	0.00	14.25	0.97	1.00	32.34	1.94	0.10	19.40	10.50
2.50	100.00	4.00	18.00	10.00	0.00	14.00	0.95	1.00	32.34	1.94	0.10	19.40	10.00
2.50	110.00	5.00	18.50	10.50	0.00	14.50	0.99	1.00	33.32	2.00	0.10	19.99	10.00
2.50	120.00	5.00	18.00	10.00	0.00	14.00	0.95	1.00	32.34	1.94	0.10	19.40	10.00
2.50	130.00	5.00	18.00	10.00	0.00	14.00	0.95	1.00	33.32	2.00	0.10	19.99	10.00
							0.96		31.93			19.16	10.92

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.50		1.00	38.00	10.00	0.00	24.00	1.63	1.00	26.34	1.58	0.10	15.80	11.50
2.50		2.00	38.00	10.00	0.00	24.00	1.63	1.00	28.36	1.70	0.10	17.02	11.50
2.50		3.00	38.00	10.00	0.00	24.00	1.63	1.00	29.14	1.75	0.10	17.48	10.50
2.50		4.00	38.00	10.00	0.00	24.00	1.63	1.00	29.62	1.78	0.10	17.77	10.00
2.50		5.00	38.00	10.00	0.00	24.00	1.63	1.00	30.25	1.82	0.10	18.15	10.00
												17.25	

SECOND CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.50			38.00	10.00	0.00	24.00	1.63	1.00	28.85	1.73	0.10	17.31	10.00

14-Mar-15	
Experiment 15	
Start UF Sample No	1028741

	REQUIRED	EXPERIMENTAL	VARIANCE
Protein concentration (g/L)	180.00	16.01	-1.99
Ionic strength (M)	2.25	2.25	0.00
Temperature (°C)	20.00	20	0.00
Ethanol Concentration (%)	3.25	2.51	-0.74
TMP (Bar)	1.00	1.14	0.14

CLEAN WATER FLUX

Hz	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	PERMEATE FLUX 20°c	TEMPERATURE	NWP (LMH/PSI)	NWP20°c (LMH/PSI)
3.90	5.00	0.00	0.00	2.50	0.17	1.00	100.00	6.00	0.10	60.00	48.60	29.00	12.00	9.72
4.50	10.00	0.00	0.00	5.00	0.34	1.00	140.00	8.40	0.10	84.00	68.04	29.00	8.40	6.80
5.00	15.00	0.00	0.00	7.50	0.51	1.00	175.00	10.50	0.10	105.00	85.05	29.00	7.00	5.67
5.50	20.00	0.00	0.00	10.00	0.68	1.00	210.00	12.60	0.10	126.00	102.06	29.00	6.30	5.10

FIRST CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.90	0.00		22.50	9.00	0.00	15.75	1.07	1.00	50.90	3.05	0.10	30.54	20.00
									50.90				

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.50	0.00	1.00	22.50	9.00	0.00	15.75	1.07	1.00	39.20	2.35	0.10	23.52	20.00
2.50	10.00	2.00	23.00	9.50	0.00	16.25	1.11	1.00	39.20	2.35	0.10	23.52	20.00
2.50	15.00	2.00	24.00	10.00	0.00	17.00	1.16	1.00	39.20	2.35	0.10	23.52	20.00
2.50	25.00	3.00	24.00	10.00	0.00	17.00	1.16	1.00	39.20	2.35	0.10	23.52	20.00
2.50	30.00	3.00	24.00	10.00	0.00	17.00	1.16	1.00	39.20	2.35	0.10	23.52	20.00
2.50	45.00	4.00	24.00	10.00	0.00	17.00	1.16	1.00	39.20	2.35	0.10	23.52	20.00
2.50	55.00	5.00	24.00	10.00	0.00	17.00	1.16	1.00	39.20	2.35	0.10	23.52	20.00
							1.14		39.20			23.52	20.00

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.50		1.00	22.50	9.00	0.00	15.75	1.07	1.00	36.45	2.19	0.10	21.87	20.00
2.50		2.00	35.00	13.00	0.00	24.00	1.63	1.00	37.74	2.26	0.10	22.64	20.00
2.50		3.00	35.00	13.00	0.00	24.00	1.63	1.00	38.12	2.29	0.10	22.87	20.00
2.50		4.00	35.00	13.00	0.00	24.00	1.63	1.00	38.27	2.30	0.10	22.96	20.00
2.50		5.00	35.00	13.00	0.00	24.00	1.63	1.00	39.01	2.34	0.10	23.41	20.00
												22.75	

SECOND CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.60			20.20	12.20	0.00	16.20	1.10	1.00	35.19	2.11	0.10	21.11	20.00

02-Apr-15	
Experiment 16	
Start UF Sample No	1028742

	REQUIRED	EXPERIMENTAL	VARIANCE
Protein concentration (g/L)	180.00	17.19	-0.81
Ionic strength (M)	2.25	2.25	0.00
Temperature (°C)	20.00	20.5	0.50
Ethanol Concentration (%)	7.75	5.89	-1.86
TMP (Bar)	1.00	1.67	0.67

CLEAN WATER FLUX

Hz	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	PERMEATE FLUX 20°c	TEMPERATURE	NWP (LMH/PSI)	NWP20°c (LMH/PSI)
3.90	5.00	0.00	0.00	2.50	0.17	1.00	100.00	6.00	0.10	60.00	53.40	25.00	12.00	10.68
4.70	10.00	0.00	0.00	5.00	0.34	1.00	140.00	8.40	0.10	84.00	74.76	25.00	8.40	7.48
5.70	15.00	0.00	0.00	7.50	0.51	1.00	188.00	11.28	0.10	112.80	100.39	25.00	7.52	6.69
6.80	20.00	0.00	0.00	10.00	0.68	1.00	238.00	14.28	0.10	142.80	127.09	25.00	7.14	6.35

FIRST CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.30	0.00		17.50	10.00	0.00	13.75	0.94	1.00	35.84	2.15	0.10	21.50	19.00
									35.84				

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.50	0.00	1.00	17.50	8.00	0.00	12.75	0.87	1.00	29.40	1.76	0.10	17.64	19.00
2.60	10.00	1.00	19.50	9.00	0.00	14.25	0.97	1.00	32.34	1.94	0.10	19.40	19.00
2.60	20.00	1.00	19.50	9.00	0.00	14.25	0.97	1.00	32.34	1.94	0.10	19.40	19.00
2.60	30.00	2.00	19.50	9.00	0.00	14.25	0.97	1.00	33.32	2.00	0.10	19.99	19.50
2.60	40.00	2.00	19.50	9.50	0.00	14.50	0.99	1.00	34.30	2.06	0.10	20.58	20.00
2.60	50.00	2.00	19.50	9.50	0.00	14.50	0.99	1.00	34.30	2.06	0.10	20.58	20.00
2.60	55.00	3.00	19.50	9.50	0.00	14.50	0.99	1.00	35.28	2.12	0.10	21.17	20.00
2.60	65.00	3.00	20.00	10.00	0.00	15.00	1.02	1.00	34.30	2.06	0.10	20.58	20.00
2.60	75.00	3.00	20.00	9.50	0.00	14.75	1.00	1.00	34.30	2.06	0.10	20.58	20.00
2.60	80.00	4.00	19.50	9.50	0.00	14.50	0.99	1.00	34.30	2.06	0.10	20.58	20.50
2.60	90.00	4.00	20.00	10.00	0.00	15.00	1.02	1.00	35.28	2.12	0.10	21.17	20.50
2.60	105.00	5.00	20.00	10.00	0.00	15.00	1.02	1.00	35.28	2.12	0.10	21.17	20.00
2.60	115.00	5.00	20.00	10.00	0.00	15.00	1.02	1.00	35.28	2.12	0.10	21.17	20.00
2.60	120.00	5.00	20.00	10.00	0.00	15.00	1.02	1.00	35.28	2.12	0.10	21.17	20.00
							0.99		33.95			20.37	19.82

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.60		1.00	19.50	9.00	0.00	14.25	0.97	1.00	27.05	1.62	0.10	16.23	20.00
2.60		2.00	19.50	9.00	0.00	14.25	0.97	1.00	30.63	1.84	0.10	18.38	20.00
2.60		3.00	20.00	9.50	0.00	14.75	1.00	1.00	31.54	1.89	0.10	18.92	20.00
2.60		4.00	20.00	10.00	0.00	15.00	1.02	1.00	31.92	1.92	0.10	19.15	20.00
2.60		5.00	20.00	10.00	0.00	15.00	1.02	1.00	32.25	1.94	0.10	19.35	20.00
												18.41	

SECOND CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.70			22.10	11.20	0.00	16.65	1.13	1.00	30.10	1.81	0.10	18.06	20.00

10-Mar-15	
Experiment 17	
Start UF Sample No	1028743

	REQUIRED	EXPERIMENTAL	VARIANCE
Protein concentration (g/L)	60.00	5.1	-0.9
Ionic strength (M)	1.50	1.5	0.00
Temperature (°C)	15.00	16	1.00
Ethanol Concentration (%)	5.50	3.73	-1.77
TMP (Bar)	1.00	1.11	0.11

CLEAN WATER FLUX

Hz	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	PERMEATE FLUX 20°c	TEMPERATURE	NWP (LMH/PSI)	NWP20°c (LMH/PSI)
4.50	5.00	0.00	0.00	2.50	0.17	1.00	125.00	7.50	0.10	75.00	63.75	27.00	15.00	12.75
5.20	10.00	0.00	0.00	5.00	0.34	1.00	175.00	10.50	0.10	105.00	89.25	27.00	10.50	8.93
5.90	15.00	0.00	0.00	7.50	0.51	1.00	215.00	12.90	0.10	129.00	109.65	27.00	8.60	7.31
6.50	20.00	0.00	0.00	10.00	0.68	1.00	230.00	13.80	0.10	138.00	117.30	27.00	6.90	5.87

FIRST CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.50	0.00		20.00	9.00	0.00	14.50	0.99	1.00	71.68	4.30	0.10	43.01	10.00
									71.68				

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.60	0.00	1.00	22.00	10.00	0.00	16.00	1.09	1.00	88.20	5.29	0.10	52.92	15.00
3.60	5.00	1.00	22.00	10.00	0.00	16.00	1.09	1.00	88.20	5.29	0.10	52.92	15.50
3.60	10.00	2.00	22.50	10.00	0.00	16.25	1.11	1.00	88.20	5.29	0.10	52.92	15.50
3.60	15.00	2.00	22.50	10.00	0.00	16.25	1.11	1.00	87.22	5.23	0.10	52.33	15.50
3.60	20.00	3.00	22.50	10.00	0.00	16.25	1.11	1.00	87.22	5.23	0.10	52.33	15.50
3.60	25.00	3.00	23.00	10.00	0.00	16.50	1.12	1.00	87.22	5.23	0.10	52.33	16.00
3.60	30.00	4.00	23.00	10.50	0.00	16.75	1.14	1.00	87.22	5.23	0.10	52.33	16.00
3.60	35.00	5.00	23.00	10.50	0.00	16.75	1.14	1.00	87.22	5.23	0.10	52.33	15.50
3.60	40.00	5.00	23.00	10.50	0.00	16.75	1.14	1.00	87.22	5.23	0.10	52.33	15.50
							1.11		87.55			52.53	15.56

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.60	0.00	1.00	22.00	10.00	0.00	16.00	1.09	1.00	88.20	5.29	0.10	52.92	15.00
3.60	5.00	1.00	22.00	10.00	0.00	16.00	1.09	1.00	88.20	5.29	0.10	52.92	15.50
												52.92	
3.60	10.00	2.00	22.50	10.00	0.00	16.25	1.11	1.00	88.20	5.29	0.10	52.92	15.50
3.60	15.00	2.00	22.50	10.00	0.00	16.25	1.11	1.00	87.22	5.23	0.10	52.33	15.50
												52.63	
3.60	20.00	3.00	22.50	10.00	0.00	16.25	1.11	1.00	87.22	5.23	0.10	52.33	15.50
3.60	25.00	3.00	23.00	10.00	0.00	16.50	1.12	1.00	87.22	5.23	0.10	52.33	16.00
												52.33	
3.60	30.00	4.00	23.00	10.50	0.00	16.75	1.14	1.00	87.22	5.23	0.10	52.33	16.00
												52.33	
3.60	35.00	5.00	23.00	10.50	0.00	16.75	1.14	1.00	87.22	5.23	0.10	52.33	15.50
3.60	40.00	5.00	23.00	10.50	0.00	16.75	1.14	1.00	87.22	5.23	0.10	52.33	15.50
							1.11		87.55			52.33	15.56

SECOND CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.60	0.00		23.00	10.50	0.00	16.75	1.14	1.00	69.67	4.18	0.10	41.80	15.00

29-Mar-15	
Experiment 18	
Start UF Sample No	1028744

	REQUIRED	EXPERIMENTAL	VARIANCE
Protein concentration (g/L)	220.00	19.58	-2.42
Ionic strength (M)	1.50	1.5	0.00
Temperature (°C)	15.00	16	1.00
Ethanol Concentration (%)	5.50		-5.50
TMP (Bar)	1.00	0.99	-0.01

CLEAN WATER FLUX

Hz	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	PERMEATE FLUX 20°c	TEMPERATURE	NWP (LMH/PSI)	NWP20°c (LMH/PSI)
3.90	5.00	0.00	0.00	2.50	0.17	1.00	75.00	4.50	0.10	45.00	39.15	26.00	9.00	7.83
4.60	10.00	0.00	0.00	5.00	0.34	1.00	119.00	7.14	0.10	71.40	62.12	26.00	7.14	6.21
5.20	15.00	0.00	0.00	7.50	0.51	1.00	159.00	9.54	0.10	95.40	83.00	26.00	6.36	5.53
5.90	20.00	0.00	0.00	10.00	0.68	1.00	202.00	12.12	0.10	121.20	105.44	26.00	6.06	5.27

FIRST CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.10	0.00		20.00	8.00	0.00	14.00	0.95	1.00	30.44	1.83	0.10	18.26	15.00
									30.44				

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.10	0.00	1.00	23.50	7.00	0.00	15.25	1.04	1.00	18.62	1.12	0.10	11.17	16.00
2.10	10.00	1.00	22.50	6.50	0.00	14.50	0.99	1.00	19.60	1.18	0.10	11.76	16.00
2.10	20.00	1.00	22.50	6.50	0.00	14.50	0.99	1.00	20.58	1.23	0.10	12.35	16.00
2.10	30.00	2.00	22.50	6.50	0.00	14.50	0.99	1.00	20.58	1.23	0.10	12.35	16.00
2.10	65.00	3.00	22.50	6.50	0.00	14.50	0.99	1.00	20.58	1.23	0.10	12.35	16.00
2.10	75.00	4.00	22.50	6.50	0.00	14.50	0.99	1.00	22.05	1.32	0.10	13.23	15.00
2.10	85.00	4.00	22.50	6.50	0.00	14.50	0.99	1.00	21.56	1.29	0.10	12.94	15.00
2.10	100.00	5.00	22.50	6.50	0.00	14.50	0.99	1.00	21.56	1.29	0.10	12.94	14.50
2.10	105.00	5.00	22.50	6.50	0.00	14.50	0.99	1.00	21.56	1.29	0.10	12.94	14.50
							0.99		20.74			12.45	15.44

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.10		1.00	22.50	6.50	0.00	14.50	0.99	1.00	19.41	1.16	0.10	11.65	16.00
2.10		2.00	22.50	6.50	0.00	14.50	0.99	1.00	19.86	1.19	0.10	11.92	16.00
2.10		3.00	22.50	6.50	0.00	14.50	0.99	1.00	20.91	1.25	0.10	12.55	15.00
2.10		4.00	22.50	6.50	0.00	14.50	0.99	1.00	21.11	1.27	0.10	12.67	15.00
2.10		5.00	22.50	6.50	0.00	14.50	0.99	1.00	21.51	1.29	0.10	12.91	14.50
												12.34	

SECOND CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
					0.00	0.00	0.00	1.00		0.00	0.10	0.00	15.00

11-Mar-15	
Experiment 19	
Start UF Sample No	1028754

	REQUIRED	EXPERIMENTAL	VARIANCE
Protein concentration (g/L)	140.00	13.67	-0.33
Ionic strength (M)	0.00	0	0.00
Temperature (°C)	15.00	16.5	1.50
Ethanol Concentration (%)	5.50	4.28	-1.22
TMP (Bar)	1.00	1.12	0.12

CLEAN WATER FLUX

Hz	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	PERMEATE FLUX 20°c	TEMPERATURE	NWP (LMH/PSI)	NWP20°c (LMH/PSI)
4.30	5.00	0.00	0.00	2.50	0.17	1.00	94.00	5.64	0.10	56.40	45.12	30.00	11.28	9.02
5.00	10.00	0.00	0.00	5.00	0.34	1.00	150.00	9.00	0.10	90.00	72.00	30.00	9.00	7.20
5.50	15.00	0.00	0.00	7.50	0.51	1.00	200.00	12.00	0.10	120.00	96.00	30.00	8.00	6.40
6.00	20.00	0.00	0.00	10.00	0.68	1.00	220.00	13.20	0.10	132.00	105.60	30.00	6.60	5.28

FIRST CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.20	0.00		22.50	10.50	0.00	16.50	1.12	1.00	51.38	3.08	0.10	30.83	13.00
									51.38				

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.10	0.00	1.00	22.50	9.00	0.00	15.75	1.07	1.00	41.16	2.47	0.10	24.70	16.50
3.10	5.00	1.00	22.50	9.00	0.00	15.75	1.07	1.00	41.16	2.47	0.10	24.70	16.50
3.10	10.00	1.00	22.50	9.00	0.00	15.75	1.07	1.00	41.16	2.47	0.10	24.70	16.50
3.10	15.00	2.00	22.50	9.50	0.00	16.00	1.09	1.00	41.16	2.47	0.10	24.70	16.00
3.10	20.00	2.00	22.50	9.00	0.00	15.75	1.07	1.00	41.16	2.47	0.10	24.70	16.50
3.10	25.00	2.00	22.50	9.00	0.00	15.75	1.07	1.00	41.16	2.47	0.10	24.70	16.50
3.10	30.00	2.00	22.50	9.00	0.00	15.75	1.07	1.00	41.16	2.47	0.10	24.70	16.50
3.10	35.00	3.00	22.50	9.00	0.00	15.75	1.07	1.00	41.16	2.47	0.10	24.70	16.00
3.10	40.00	3.00	22.50	9.00	0.00	15.75	1.07	1.00	41.16	2.47	0.10	24.70	16.00
3.10	45.00	3.00	22.50	10.00	0.00	16.25	1.11	1.00	40.18	2.41	0.10	24.11	16.00
3.10	50.00	3.00	24.00	10.00	0.00	17.00	1.16	1.00	40.18	2.41	0.10	24.11	16.00
3.10	55.00	4.00	24.00	10.00	0.00	17.00	1.16	1.00	40.18	2.41	0.10	24.11	16.00
3.10	60.00	4.00	24.00	10.00	0.00	17.00	1.16	1.00	39.20	2.35	0.10	23.52	16.00
3.10	65.00	4.00	24.00	10.00	0.00	17.00	1.16	1.00	40.18	2.41	0.10	24.11	16.00
3.10	70.00	4.00	24.00	10.00	0.00	17.00	1.16	1.00	40.18	2.41	0.10	24.11	16.00
3.10	75.00	5.00	24.00	10.00	0.00	17.00	1.16	1.00	40.18	2.41	0.10	24.11	15.50
3.10	80.00	5.00	24.00	10.00	0.00	17.00	1.16	1.00	41.16	2.47	0.10	24.70	15.50
3.10	85.00	5.00	24.00	10.00	0.00	17.00	1.16	1.00	40.18	2.41	0.10	24.11	15.50
3.10	90.00	5.00	24.50	10.50	0.00	17.50	1.19	1.00	41.16	2.47	0.10	24.70	15.00
							1.12		40.70			24.42	16.03

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.10	0.00	1.00	22.50	9.00	0.00	15.75	1.07	1.00	41.16	2.47	0.10	24.70	16.50
3.10	5.00	1.00	22.50	9.00	0.00	15.75	1.07	1.00	41.16	2.47	0.10	24.70	16.50
3.10	10.00	1.00	22.50	9.00	0.00	15.75	1.07	1.00	41.16	2.47	0.10	24.70	16.50
												24.70	
3.10	15.00	2.00	22.50	9.50	0.00	16.00	1.09	1.00	41.16	2.47	0.10	24.70	16.00
3.10	20.00	2.00	22.50	9.00	0.00	15.75	1.07	1.00	41.16	2.47	0.10	24.70	16.50
3.10	25.00	2.00	22.50	9.00	0.00	15.75	1.07	1.00	41.16	2.47	0.10	24.70	16.50
3.10	30.00	2.00	22.50	9.00	0.00	15.75	1.07	1.00	41.16	2.47	0.10	24.70	16.50
												24.70	
3.10	35.00	3.00	22.50	9.00	0.00	15.75	1.07	1.00	41.16	2.47	0.10	24.70	16.00
3.10	40.00	3.00	22.50	9.00	0.00	15.75	1.07	1.00	41.16	2.47	0.10	24.70	16.00
3.10	45.00	3.00	22.50	10.00	0.00	16.25	1.11	1.00	40.18	2.41	0.10	24.11	16.00
3.10	50.00	3.00	24.00	10.00	0.00	17.00	1.16	1.00	40.18	2.41	0.10	24.11	16.00
												24.40	
3.10	55.00	4.00	24.00	10.00	0.00	17.00	1.16	1.00	40.18	2.41	0.10	24.11	16.00
3.10	60.00	4.00	24.00	10.00	0.00	17.00	1.16	1.00	39.20	2.35	0.10	23.52	16.00
3.10	65.00	4.00	24.00	10.00	0.00	17.00	1.16	1.00	40.18	2.41	0.10	24.11	16.00
3.10	70.00	4.00	24.00	10.00	0.00	17.00	1.16	1.00	40.18	2.41	0.10	24.11	16.00
												23.96	
3.10	75.00	5.00	24.00	10.00	0.00	17.00	1.16	1.00	40.18	2.41	0.10	24.11	15.50
3.10	80.00	5.00	24.00	10.00	0.00	17.00	1.16	1.00	41.16	2.47	0.10	24.70	15.50
3.10	85.00	5.00	24.00	10.00	0.00	17.00	1.16	1.00	40.18	2.41	0.10	24.11	15.50
3.10	90.00	5.00	24.50	10.50	0.00	17.50	1.19	1.00	41.16	2.47	0.10	24.70	15.00
							1.12		40.70			24.40	16.03

SECOND CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.00	0.00		23.00	9.00	0.00	16.00	1.09	1.00	34.86	2.09	0.10	20.92	15.50

23-Mar-15	
Experiment 20	
Start UF Sample No	1028746

	REQUIRED	EXPERIMENTAL	VARIANCE
Protein concentration (g/L)	140.00	12.23	-1.77
Ionic strength (M)	3.00	3	0.00
Temperature (°C)	15.00	16	1.00
Ethanol Concentration (%)	5.50	4.17	-1.33
TMP (Bar)	1.00	1.2	0.20

CLEAN WATER FLUX

Hz	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	PERMEATE FLUX 20°c	TEMPERATURE	NWP (LMH/PSI)	NWP20°c (LMH/PSI)
3.80	5.00	0.00	0.00	2.50	0.17	1.00	76.00	4.56	0.10	45.60	38.30	27.50	9.12	7.66
4.60	10.00	0.00	0.00	5.00	0.34	1.00	120.00	7.20	0.10	72.00	60.48	27.50	7.20	6.05
5.20	15.00	0.00	0.00	7.50	0.51	1.00	165.00	9.90	0.10	99.00	83.16	27.50	6.60	5.54
5.80	20.00	0.00	0.00	10.00	0.68	1.00	210.00	12.60	0.10	126.00	105.84	27.50	6.30	5.29

FIRST CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.40	0.00		24.50	10.00	0.00	17.25	1.17	1.00	51.04	3.06	0.10	30.62	15.00
									51.04				

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.20	0.00	1.00	24.00	9.50	0.00	16.75	1.14	1.00	49.00	2.94	0.10	29.40	16.00
3.20	5.00	1.00	24.00	9.50	0.00	16.75	1.14	1.00	49.00	2.94	0.10	29.40	16.00
3.20	10.00	1.00	25.00	10.00	0.00	17.50	1.19	1.00	49.98	3.00	0.10	29.99	16.00
3.20	15.00	2.00	25.50	10.50	0.00	18.00	1.22	1.00	50.96	3.06	0.10	30.58	16.00
3.10	20.00	2.00	25.00	9.50	0.00	17.25	1.17	1.00	47.04	2.82	0.10	28.22	16.00
3.10	25.00	2.00	25.00	9.50	0.00	17.25	1.17	1.00	48.02	2.88	0.10	28.81	16.00
3.10	30.00	3.00	26.00	10.00	0.00	18.00	1.22	1.00	49.00	2.94	0.10	29.40	16.00
3.10	35.00	3.00	26.00	10.00	0.00	18.00	1.22	1.00	49.00	2.94	0.10	29.40	16.00
3.10	40.00	3.00	26.00	10.00	0.00	18.00	1.22	1.00	49.00	2.94	0.10	29.40	16.00
3.10	45.00	4.00	26.00	10.00	0.00	18.00	1.22	1.00	49.00	2.94	0.10	29.40	16.00
3.10	50.00	4.00	26.00	10.00	0.00	18.00	1.22	1.00	49.00	2.94	0.10	29.40	16.00
3.10	55.00	4.00	26.00	10.00	0.00	18.00	1.22	1.00	49.00	2.94	0.10	29.40	16.00
3.10	60.00	5.00	26.00	10.00	0.00	18.00	1.22	1.00	49.00	2.94	0.10	29.40	16.00
3.10	65.00	5.00	26.00	10.00	0.00	18.00	1.22	1.00	49.00	2.94	0.10	29.40	16.00
3.10	70.00	5.00	26.00	10.00	0.00	18.00	1.22	1.00	49.00	2.94	0.10	29.40	16.00
							1.20		49.00			29.40	16.00

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.20	0.00	1.00	24.00	9.50	0.00	16.75	1.14	1.00	49.00	2.94	0.10	29.40	16.00
3.20	5.00	1.00	24.00	9.50	0.00	16.75	1.14	1.00	49.00	2.94	0.10	29.40	16.00
3.20	10.00	1.00	25.00	10.00	0.00	17.50	1.19	1.00	49.98	3.00	0.10	29.99	16.00
												29.60	
3.20	15.00	2.00	25.50	10.50	0.00	18.00	1.22	1.00	50.96	3.06	0.10	30.58	16.00
3.10	20.00	2.00	25.00	9.50	0.00	17.25	1.17	1.00	47.04	2.82	0.10	28.22	16.00
3.10	25.00	2.00	25.00	9.50	0.00	17.25	1.17	1.00	48.02	2.88	0.10	28.81	16.00
												29.20	
3.10	30.00	3.00	26.00	10.00	0.00	18.00	1.22	1.00	49.00	2.94	0.10	29.40	16.00
3.10	35.00	3.00	26.00	10.00	0.00	18.00	1.22	1.00	49.00	2.94	0.10	29.40	16.00
3.10	40.00	3.00	26.00	10.00	0.00	18.00	1.22	1.00	49.00	2.94	0.10	29.40	16.00
												29.40	
3.10	45.00	4.00	26.00	10.00	0.00	18.00	1.22	1.00	49.00	2.94	0.10	29.40	16.00
3.10	50.00	4.00	26.00	10.00	0.00	18.00	1.22	1.00	49.00	2.94	0.10	29.40	16.00
3.10	55.00	4.00	26.00	10.00	0.00	18.00	1.22	1.00	49.00	2.94	0.10	29.40	16.00
												29.40	
3.10	60.00	5.00	26.00	10.00	0.00	18.00	1.22	1.00	49.00	2.94	0.10	29.40	16.00
3.10	65.00	5.00	26.00	10.00	0.00	18.00	1.22	1.00	49.00	2.94	0.10	29.40	16.00
3.10	70.00	5.00	26.00	10.00	0.00	18.00	1.22	1.00	49.00	2.94	0.10	29.40	16.00
							1.20		49.00			29.40	16.00

SECOND CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.10	0.00		26.00	10.00	0.00	18.00	1.22	1.00	41.48	2.49	0.10	24.89	16.00

27-Mar-15	
Experiment 21	
Start UF Sample No	1028747

	REQUIRED	EXPERIMENTAL	VARIANCE
Protein concentration (g/L)	140.00		-14
Ionic strength (M)	1.50	1.5	0.00
Temperature (°C)	5.00	7	2.00
Ethanol Concentration (%)	5.50		-5.50
TMP (Bar)	1.00		-1.00

CLEAN WATER FLUX

Hz	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	PERMEATE FLUX 20°c	TEMPERATURE	NWP (LMH/PSI)	NWP20°c (LMH/PSI)
3.80	5.00	0.00	0.00	2.50	0.17	1.00	75.00	4.50	0.10	45.00	40.95	24.00	9.00	8.19
4.70	10.00	0.00	0.00	5.00	0.34	1.00	118.00	7.08	0.10	70.80	64.43	24.00	7.08	6.44
5.40	15.00	0.00	0.00	7.50	0.51	1.00	160.00	9.60	0.10	96.00	87.36	24.00	6.40	5.82
6.00	20.00	0.00	0.00	10.00	0.68	1.00	205.00	12.30	0.10	123.00	111.93	24.00	6.15	5.60

FIRST CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
	0.00				0.00	0.00	0.00	1.00		0.00	0.10	0.00	
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DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.90	0.00	1.00	20.00	10.00	0.00	15.00	1.02	1.00	44.10	2.65	0.10	26.46	7.00
2.90	5.00	1.00	20.00	10.00	0.00	15.00	1.02	1.00	46.06	2.76	0.10	27.64	7.00
2.90	20.00	2.00	20.00	10.00	0.00	15.00	1.02	1.00	47.04	2.82	0.10	28.22	7.00
2.90	25.00	2.00	20.00	10.00	0.00	15.00	1.02	1.00	47.04	2.82	0.10	28.22	6.50
2.90	35.00	3.00	20.00	10.00	0.00	15.00	1.02	1.00	48.02	2.88	0.10	28.81	6.50
2.90	40.00	3.00	20.00	10.00	0.00	15.00	1.02	1.00	48.02	2.88	0.10	28.81	6.50
2.90	45.00	3.00	20.00	10.00	0.00	15.00	1.02	1.00	49.00	2.94	0.10	29.40	6.50
2.90	55.00	4.00	20.00	10.00	0.00	15.00	1.02	1.00	49.98	3.00	0.10	29.99	6.50
2.90	60.00	4.00	20.00	10.00	0.00	15.00	1.02	1.00	50.96	3.06	0.10	30.58	6.50
2.90	65.00	5.00	20.00	10.00	0.00	15.00	1.02	1.00	50.96	3.06	0.10	30.58	6.00
2.90	75.00	5.00	20.00	10.00	0.00	15.00	1.02	1.00	51.94	3.12	0.10	31.16	6.00
							1.02		48.47			29.08	6.55

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.40		1.00	40.00	10.00	0.00	25.00	1.70	1.00	41.55	2.49	0.10	24.93	20.00
2.40		2.00	40.00	10.00	0.00	25.00	1.70	1.00	45.55	2.73	0.10	27.33	20.00
2.40		3.00	40.00	10.00	0.00	25.00	1.70	1.00	46.63	2.80	0.10	27.98	20.00
2.40		4.00	40.00	10.00	0.00	25.00	1.70	1.00	48.83	2.93	0.10	29.30	20.00
2.40		5.00	40.00	10.00	0.00	25.00	1.70	1.00	50.45	3.03	0.10	30.27	20.00
												27.96	

SECOND CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
				10.50	0.00	5.25	0.36	1.00		0.00	0.10	0.00	

09-Mar-15	
Experiment 22	
Start UF Sample No	1028748

	REQUIRED	EXPERIMENTAL	VARIANCE
Protein concentration (g/L)	140.00	12.26	-1.74
Ionic strength (M)	1.50	1.5	0.00
Temperature (°C)	25.00	26	1.00
Ethanol Concentration (%)	5.50	4.32	-1.18
TMP (Bar)	1.00	1.11	0.11

CLEAN WATER FLUX

Hz	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	PERMEATE FLUX 20°c	TEMPERATURE	NWP (LMH/PSI)	NWP20°c (LMH/PSI)
3.90	5.00	0.00	0.00	2.50	0.17	1.00	71.00	4.26	0.10	42.60	36.21	27.00	8.52	7.24
4.60	10.00	0.00	0.00	5.00	0.34	1.00	120.00	7.20	0.10	72.00	61.20	27.00	7.20	6.12
5.20	15.00	0.00	0.00	7.50	0.51	1.00	162.00	9.72	0.10	97.20	82.62	27.00	6.48	5.51
5.80	20.00	0.00	0.00	10.00	0.68	1.00	200.00	12.00	0.10	120.00	102.00	27.00	6.00	5.10

FIRST CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.40	0.00		24.50	9.50	0.00	17.00	1.16	1.00	62.92	3.78	0.10	37.75	10.00
									62.92				

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.20	0.00	1.00	23.50	9.00	0.00	16.25	1.11	1.00	54.88	3.29	0.10	32.93	24.00
3.20	5.00	1.00	23.50	9.00	0.00	16.25	1.11	1.00	56.84	3.41	0.10	34.10	24.50
3.20	10.00	1.00	23.50	9.00	0.00	16.25	1.11	1.00	56.84	3.41	0.10	34.10	24.50
3.20	15.00	2.00	23.50	9.00	0.00	16.25	1.11	1.00	56.84	3.41	0.10	34.10	24.00
3.20	20.00	2.00	23.50	9.00	0.00	16.25	1.11	1.00	56.84	3.41	0.10	34.10	24.00
3.20	25.00	2.00	23.50	9.00	0.00	16.25	1.11	1.00	56.84	3.41	0.10	34.10	24.50
3.20	30.00	3.00	23.50	9.00	0.00	16.25	1.11	1.00	56.84	3.41	0.10	34.10	25.00
3.20	35.00	3.00	23.50	9.00	0.00	16.25	1.11	1.00	57.82	3.47	0.10	34.69	25.00
3.20	40.00	4.00	23.50	9.00	0.00	16.25	1.11	1.00	57.82	3.47	0.10	34.69	25.00
3.20	45.00	4.00	23.50	9.00	0.00	16.25	1.11	1.00	57.82	3.47	0.10	34.69	25.50
3.20	50.00	4.00	23.50	9.00	0.00	16.25	1.11	1.00	57.82	3.47	0.10	34.69	25.50
3.20	55.00	5.00	23.50	9.00	0.00	16.25	1.11	1.00	57.82	3.47	0.10	34.69	25.50
3.20	60.00	5.00	23.50	9.00	0.00	16.25	1.11	1.00	57.82	3.47	0.10	34.69	26.00
3.20	65.00	5.00	23.50	9.00	0.00	16.25	1.11	1.00	57.82	3.47	0.10	34.69	25.50
							1.11		57.14			34.31	24.89

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.20	0.00	1.00	23.50	9.00	0.00	16.25	1.11	1.00	54.88	3.29	0.10	32.93	24.00
3.20	5.00	1.00	23.50	9.00	0.00	16.25	1.11	1.00	56.84	3.41	0.10	34.10	24.50
3.20	10.00	1.00	23.50	9.00	0.00	16.25	1.11	1.00	56.84	3.41	0.10	34.10	24.50
												33.71	
3.20	15.00	2.00	23.50	9.00	0.00	16.25	1.11	1.00	56.84	3.41	0.10	34.10	24.00
3.20	20.00	2.00	23.50	9.00	0.00	16.25	1.11	1.00	56.84	3.41	0.10	34.10	24.00
3.20	25.00	2.00	23.50	9.00	0.00	16.25	1.11	1.00	56.84	3.41	0.10	34.10	24.50
												34.10	
3.20	30.00	3.00	23.50	9.00	0.00	16.25	1.11	1.00	56.84	3.41	0.10	34.10	25.00
3.20	35.00	3.00	23.50	9.00	0.00	16.25	1.11	1.00	57.82	3.47	0.10	34.69	25.00
												34.40	
3.20	40.00	4.00	23.50	9.00	0.00	16.25	1.11	1.00	57.82	3.47	0.10	34.69	25.00
3.20	45.00	4.00	23.50	9.00	0.00	16.25	1.11	1.00	57.82	3.47	0.10	34.69	25.50
3.20	50.00	4.00	23.50	9.00	0.00	16.25	1.11	1.00	57.82	3.47	0.10	34.69	25.50
												34.69	
3.20	55.00	5.00	23.50	9.00	0.00	16.25	1.11	1.00	57.82	3.47	0.10	34.69	25.50
3.20	60.00	5.00	23.50	9.00	0.00	16.25	1.11	1.00	57.82	3.47	0.10	34.69	26.00
3.20	65.00	5.00	23.50	9.00	0.00	16.25	1.11	1.00	57.82	3.47	0.10	34.69	25.50
							1.11		57.14			34.69	24.89

SECOND CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.00	0.00		25.00	9.50	0.00	17.25	1.17	1.00	52.96	3.18	0.10	31.78	10.00

04-Mar-15	
Experiment 23	
Start UF Sample No	1028749

	REQUIRED	EXPERIMENTAL	VARIANCE
Protein concentration (g/L)	140.00	12.63	-1.37
Ionic strength (M)	1.50	1.5	0.00
Temperature (°C)	15.00	17	2.00
Ethanol Concentration (%)	1.00	0.23	-0.77
TMP (Bar)	1.00	1.19	0.19

CLEAN WATER FLUX

Hz	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	PERMEATE FLUX 20°c	TEMPERATURE	NWP (LMH/PSI)	NWP20°c (LMH/PSI)
3.90	5.00	0.00	0.00	2.50	0.17	1.00	100.00	6.00	0.10	60.00	52.20	26.00	12.00	10.44
4.60	10.00	0.00	0.00	5.00	0.34	1.00	145.00	8.70	0.10	87.00	75.69	26.00	8.70	7.57
5.10	15.00	0.00	0.00	7.50	0.51	1.00	185.00	11.10	0.10	111.00	96.57	26.00	7.40	6.44
5.50	20.00	0.00	0.00	10.00	0.68	1.00	220.00	13.20	0.10	132.00	114.84	26.00	6.60	5.74

FIRST CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.30	0.00		22.50	9.50	0.00	16.00	1.09	1.00	61.26	3.68	0.10	36.76	15.00
									61.26				

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.40	0.00	1.00	22.50	9.50	0.00	16.00	1.09	1.00	60.76	3.65	0.10	36.46	17.00
3.40	5.00	1.00	25.00	10.00	0.00	17.50	1.19	1.00	59.78	3.59	0.10	35.87	17.00
3.40	10.00	2.00	26.00	10.00	0.00	18.00	1.22	1.00	58.80	3.53	0.10	35.28	17.00
3.30	15.00	2.00	24.00	9.50	0.00	16.75	1.14	1.00	54.88	3.29	0.10	32.93	17.00
3.30	20.00	2.00	25.00	9.50	0.00	17.25	1.17	1.00	54.88	3.29	0.10	32.93	16.50
3.30	25.00	3.00	25.00	9.50	0.00	17.25	1.17	1.00	54.88	3.29	0.10	32.93	16.50
3.30	30.00	3.00	26.00	9.50	0.00	17.75	1.21	1.00	54.88	3.29	0.10	32.93	16.00
3.30	35.00	4.00	26.00	10.00	0.00	18.00	1.22	1.00	54.88	3.29	0.10	32.93	15.00
3.30	40.00	4.00	26.00	10.00	0.00	18.00	1.22	1.00	54.88	3.29	0.10	32.93	15.00
3.30	45.00	4.00	27.00	10.00	0.00	18.50	1.26	1.00	56.84	3.41	0.10	34.10	15.00
3.20	50.00	5.00	25.00	9.50	0.00	17.25	1.17	1.00	52.92	3.18	0.10	31.75	15.00
3.20	55.00	5.00	25.00	9.50	0.00	17.25	1.17	1.00	54.88	3.29	0.10	32.93	15.00
3.20	60.00	5.00	25.00	9.50	0.00	17.25	1.17	1.00	54.88	3.29	0.10	32.93	15.00
							1.19		56.01			33.61	15.92

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.40	0.00	1.00	22.50	9.50	0.00	16.00	1.09	1.00	60.76	3.65	0.10	36.46	17.00
3.40	5.00	1.00	25.00	10.00	0.00	17.50	1.19	1.00	59.78	3.59	0.10	35.87	17.00
												36.16	
3.40	15.00	2.00	26.00	10.00	0.00	18.00	1.22	1.00	58.80	3.53	0.10	35.28	17.00
3.30	20.00	2.00	24.00	9.50	0.00	16.75	1.14	1.00	54.88	3.29	0.10	32.93	17.00
3.30	25.00	2.00	25.00	9.50	0.00	17.25	1.17	1.00	54.88	3.29	0.10	32.93	16.50
												33.71	
3.30	30.00	3.00	25.00	9.50	0.00	17.25	1.17	1.00	54.88	3.29	0.10	32.93	16.50
3.30	35.00	3.00	26.00	9.50	0.00	17.75	1.21	1.00	54.88	3.29	0.10	32.93	16.00
												32.93	
3.30	40.00	4.00	26.00	10.00	0.00	18.00	1.22	1.00	54.88	3.29	0.10	32.93	15.00
3.30	45.00	4.00	26.00	10.00	0.00	18.00	1.22	1.00	54.88	3.29	0.10	32.93	15.00
3.30	50.00	4.00	27.00	10.00	0.00	18.50	1.26	1.00	56.84	3.41	0.10	34.10	15.00
												33.32	
3.20	55.00	5.00	25.00	9.50	0.00	17.25	1.17	1.00	52.92	3.18	0.10	31.75	15.00
3.20	60.00	5.00	25.00	9.50	0.00	17.25	1.17	1.00	54.88	3.29	0.10	32.93	15.00
3.20	65.00	5.00	25.00	9.50	0.00	17.25	1.17	1.00	54.88	3.29	0.10	32.93	15.00
												32.54	

SECOND CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.00	0.00		25.00	9.50	0.00	17.25	1.17	1.00	44.81	2.69	0.10	26.89	10.00

31-Mar-15	
Experiment 24	
Start UF Sample No	1028750

	REQUIRED	EXPERIMENTAL	VARIANCE
Protein concentration (g/L)	140.00	12	-2
Ionic strength (M)	1.50	1.5	0.00
Temperature (°C)	15.00	17	2.00
Ethanol Concentration (%)	10.00	7.02	-2.98
TMP (Bar)	1.00	1.5	0.50

CLEAN WATER FLUX

Hz	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	PERMEATE FLUX 20°c	TEMPERATURE	NWP (LMH/PSI)	NWP20°c (LMH/PSI)
3.00	5.00	0.00	0.00	2.50	0.17	1.00	62.00	3.72	0.10	37.20	32.36	26.00	7.44	6.47
4.00	10.00	0.00	0.00	5.00	0.34	1.00	91.00	5.46	0.10	54.60	47.50	26.00	5.46	4.75
4.70	15.00	0.00	0.00	7.50	0.51	1.00	120.00	7.20	0.10	72.00	62.64	26.00	4.80	4.18
5.10	20.00	0.00	0.00	10.00	0.68	1.00	148.00	8.88	0.10	88.80	77.26	26.00	4.44	3.86

FIRST CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.80	0.00		25.00	12.50	0.00	18.75	1.28	1.00	52.19	3.13	0.10	31.31	15.00
									52.19				

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.00	0.00	1.00	25.00	10.00	0.00	17.50	1.19	1.00	45.08	2.70	0.10	27.05	17.00
3.00	0.00	1.00	25.00	10.00	0.00	17.50	1.19	1.00	45.08	2.70	0.10	27.05	16.00
3.00	10.00	1.00	25.00	10.00	0.00	17.50	1.19	1.00	46.06	2.76	0.10	27.64	16.00
3.00	15.00	1.00	25.00	10.00	0.00	17.50	1.19	1.00	47.04	2.82	0.10	28.22	15.50
3.00	22.00	2.00	25.00	10.00	0.00	17.50	1.19	1.00	47.04	2.82	0.10	28.22	15.00
3.00	30.00	2.00	24.00	10.00	0.00	17.00	1.16	1.00	49.00	2.94	0.10	29.40	15.00
3.00	40.00	3.00	24.00	10.00	0.00	17.00	1.16	1.00	49.00	2.94	0.10	29.40	15.00
3.00	50.00	3.00	23.50	10.00	0.00	16.75	1.14	1.00	50.96	3.06	0.10	30.58	15.00
3.00	55.00	4.00	23.50	10.00	0.00	16.75	1.14	1.00	49.98	3.00	0.10	29.99	15.00
3.00	60.00	4.00	23.50	10.00	0.00	16.75	1.14	1.00	49.98	3.00	0.10	29.99	15.00
3.00	65.00	4.00	23.50	10.00	0.00	16.75	1.14	1.00	50.96	3.06	0.10	30.58	15.00
3.00	75.00	5.00	23.50	10.00	0.00	16.75	1.14	1.00	50.96	3.06	0.10	30.58	15.00
3.00	80.00	5.00	23.50	10.00	0.00	16.75	1.14	1.00	49.98	3.00	0.10	29.99	15.50
3.00	85.00	5.00	23.50	10.00	0.00	16.75	1.14	1.00	49.98	3.00	0.10	29.99	15.50
							1.16		48.65			29.19	15.39

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.00		1.00	35.00	10.00	0.00	22.50	1.53	1.00	41.54	2.49	0.10	24.92	16.00
3.00		2.00	34.50	10.00	0.00	22.25	1.51	1.00	45.40	2.72	0.10	27.24	15.00
3.00		3.00	33.50	10.00	0.00	21.75	1.48	1.00	46.88	2.81	0.10	28.13	15.00
3.00		4.00	33.50	10.00	0.00	21.75	1.48	1.00	48.07	2.88	0.10	28.84	15.00
3.00		5.00	33.50	10.00	0.00	21.75	1.48	1.00	48.77	2.93	0.10	29.26	15.50
												27.68	

SECOND CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.00			33.50	10.00	0.00	21.75	1.48	1.00	45.00	2.70	0.10	27.00	15.50

13-Mar-15	
Experiment 25	
Start UF Sample No	1028751

	REQUIRED	EXPERIMENTAL	VARIANCE
Protein concentration (g/L)	140.00	12.06	-1.94
Ionic strength (M)	1.50	1.5	0.00
Temperature (°C)	15.00	16.5	1.50
Ethanol Concentration (%)	5.50	4.6	-0.90
TMP (Bar)	1.00	1.14	0.14

CLEAN WATER FLUX

Hz	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	PERMEATE FLUX 20°c	TEMPERATURE	NWP (LMH/PSI)	NWP20°c (LMH/PSI)
3.90	5.00	0.00	0.00	2.50	0.17	1.00	86.00	5.16	0.10	51.60	44.89	26.00	10.32	8.98
4.20	10.00	0.00	0.00	5.00	0.34	1.00	120.00	7.20	0.10	72.00	62.64	26.00	7.20	6.26
5.50	15.00	0.00	0.00	7.50	0.51	1.00	165.00	9.90	0.10	99.00	86.13	26.00	6.60	5.74
5.90	20.00	0.00	0.00	10.00	0.68	1.00	205.00	12.30	0.10	123.00	107.01	26.00	6.15	5.35

FIRST CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.30	0.00		25.50	10.50	0.00	18.00	1.22	1.00	60.40	3.62	0.10	36.24	15.00
									60.40				

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.10	0.00	1.00	24.00	9.50	0.00	16.75	1.14	1.00	50.96	3.06	0.10	30.58	16.00
3.10	5.00	1.00	24.00	9.50	0.00	16.75	1.14	1.00	51.94	3.12	0.10	31.16	16.50
3.10	10.00	1.00	24.00	9.50	0.00	16.75	1.14	1.00	52.92	3.18	0.10	31.75	16.00
3.10	15.00	2.00	24.00	9.50	0.00	16.75	1.14	1.00	52.92	3.18	0.10	31.75	16.00
3.10	20.00	2.00	24.00	9.50	0.00	16.75	1.14	1.00	52.92	3.18	0.10	31.75	15.50
3.10	25.00	2.00	24.00	10.00	0.00	17.00	1.16	1.00	52.92	3.18	0.10	31.75	16.00
3.10	30.00	3.00	24.00	9.50	0.00	16.75	1.14	1.00	52.92	3.18	0.10	31.75	15.50
3.10	35.00	3.00	24.00	9.50	0.00	16.75	1.14	1.00	52.92	3.18	0.10	31.75	16.00
3.10	40.00	3.00	24.00	9.50	0.00	16.75	1.14	1.00	52.92	3.18	0.10	31.75	16.00
3.10	45.00	4.00	24.00	9.50	0.00	16.75	1.14	1.00	52.92	3.18	0.10	31.75	16.00
3.10	50.00	4.00	24.00	9.50	0.00	16.75	1.14	1.00	52.92	3.18	0.10	31.75	16.00
3.10	55.00	4.00	24.00	9.50	0.00	16.75	1.14	1.00	52.92	3.18	0.10	31.75	16.00
3.10	60.00	5.00	24.00	9.50	0.00	16.75	1.14	1.00	52.92	3.18	0.10	31.75	16.00
3.10	65.00	5.00	24.00	9.50	0.00	16.75	1.14	1.00	52.92	3.18	0.10	31.75	16.00
3.10	70.00	5.00	24.00	9.50	0.00	16.75	1.14	1.00	52.92	3.18	0.10	31.75	16.00
							1.14		52.72			31.63	15.97

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.10	0.00	1.00	24.00	9.50	0.00	16.75	1.14	1.00	50.96	3.06	0.10	30.58	16.00
3.10	5.00	1.00	24.00	9.50	0.00	16.75	1.14	1.00	51.94	3.12	0.10	31.16	16.50
3.10	10.00	1.00	24.00	9.50	0.00	16.75	1.14	1.00	52.92	3.18	0.10	31.75	16.00
												31.16	
3.10	15.00	2.00	24.00	9.50	0.00	16.75	1.14	1.00	52.92	3.18	0.10	31.75	16.00
3.10	20.00	2.00	24.00	9.50	0.00	16.75	1.14	1.00	52.92	3.18	0.10	31.75	15.50
3.10	25.00	2.00	24.00	9.50	0.00	16.75	1.14	1.00	52.92	3.18	0.10	31.75	16.00
												31.75	
3.10	30.00	3.00	24.00	9.50	0.00	16.75	1.14	1.00	52.92	3.18	0.10	31.75	15.50
3.10	35.00	3.00	24.00	9.50	0.00	16.75	1.14	1.00	52.92	3.18	0.10	31.75	16.00
3.10	40.00	3.00	24.00	9.50	0.00	16.75	1.14	1.00	52.92	3.18	0.10	31.75	16.00
												31.75	
3.10	45.00	4.00	24.00	9.50	0.00	16.75	1.14	1.00	52.92	3.18	0.10	31.75	16.00
3.10	50.00	4.00	24.00	9.50	0.00	16.75	1.14	1.00	52.92	3.18	0.10	31.75	16.00
3.10	55.00	4.00	24.00	9.50	0.00	16.75	1.14	1.00	52.92	3.18	0.10	31.75	16.00
												31.75	
3.10	60.00	5.00	24.00	9.50	0.00	16.75	1.14	1.00	52.92	3.18	0.10	31.75	16.00
3.10	65.00	5.00	24.00	9.50	0.00	16.75	1.14	1.00	52.92	3.18	0.10	31.75	16.00
3.10	70.00	5.00	24.00	9.50	0.00	16.75	1.14	1.00	52.92	3.18	0.10	31.75	16.00
							1.14		52.72			31.75	15.97

SECOND CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.80			24.00	9.50	0.00	16.75	1.14	1.00	46.16	2.77	0.10	27.70	16.00

09-Mar-15	
Experiment 26	
Start UF Sample No	1028752

	REQUIRED	EXPERIMENTAL	VARIANCE
Protein concentration (g/L)	140.00	12.24	-1.76
Ionic strength (M)	1.50	1.5	0.00
Temperature (°C)	15.00		-15.00
Ethanol Concentration (%)	5.50	4.33	-1.17
TMP (Bar)	1.00	1.18	0.18

CLEAN WATER FLUX

Hz	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	PERMEATE FLUX 20°c	TEMPERATURE	NWP (LMH/PSI)	NWP20°c (LMH/PSI)
3.90	5.00	0.00	0.00	2.50	0.17	1.00	81.00	4.86	0.10	48.60	40.34	28.00	9.72	8.07
4.80	10.00	0.00	0.00	5.00	0.34	1.00	130.00	7.80	0.10	78.00	64.74	28.00	7.80	6.47
5.30	15.00	0.00	0.00	7.50	0.51	1.00	180.00	10.80	0.10	108.00	89.64	28.00	7.20	5.98
5.80	20.00	0.00	0.00	10.00	0.68	1.00	219.00	13.14	0.10	131.40	109.06	28.00	6.57	5.45

FIRST CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
2.90	0.00		24.00	9.50	0.00	16.75	1.14	1.00	50.02	3.00	0.10	30.01	15.00
									50.02				

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.20	0.00	1.00	24.50	9.00	0.00	16.75	1.14	1.00	49.00	2.94	0.10	29.40	16.00
3.20	5.00	1.00	24.50	9.00	0.00	16.75	1.14	1.00	49.98	3.00	0.10	29.99	16.50
3.20	10.00	1.00	25.00	9.50	0.00	17.25	1.17	1.00	50.96	3.06	0.10	30.58	16.50
3.20	15.00	2.00	25.00	9.50	0.00	17.25	1.17	1.00	50.96	3.06	0.10	30.58	16.00
3.20	20.00	2.00	25.00	9.50	0.00	17.25	1.17	1.00	51.94	3.12	0.10	31.16	16.00
3.20	25.00	2.00	25.00	9.50	0.00	17.25	1.17	1.00	51.94	3.12	0.10	31.16	16.00
3.20	30.00	3.00	25.00	9.50	0.00	17.25	1.17	1.00	51.94	3.12	0.10	31.16	16.00
3.20	35.00	3.00	25.00	9.50	0.00	17.25	1.17	1.00	51.94	3.12	0.10	31.16	16.00
3.20	40.00	3.00	25.00	9.50	0.00	17.25	1.17	1.00	51.94	3.12	0.10	31.16	16.00
3.20	45.00	4.00	25.00	9.50	0.00	17.25	1.17	1.00	51.94	3.12	0.10	31.16	16.00
3.20	50.00	4.00	25.00	9.50	0.00	17.25	1.17	1.00	52.92	3.18	0.10	31.75	16.00
3.20	55.00	4.00	25.00	9.50	0.00	17.25	1.17	1.00	51.94	3.12	0.10	31.16	16.00
3.20	60.00	5.00	25.50	10.00	0.00	17.75	1.21	1.00	51.94	3.12	0.10	31.16	16.00
3.20	65.00	5.00	25.50	10.00	0.00	17.75	1.21	1.00	52.92	3.18	0.10	31.75	16.00
3.20	70.00	5.00	25.50	10.00	0.00	17.75	1.21	1.00	52.92	3.18	0.10	31.75	16.00
							1.18		51.49			31.01	16.07

DIAFILTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.20	0.00	1.00	24.50	9.00	0.00	16.75	1.14	1.00	49.00	2.94	0.10	29.40	16.00
3.20	5.00	1.00	24.50	9.00	0.00	16.75	1.14	1.00	49.98	3.00	0.10	29.99	16.50
3.20	10.00	1.00	25.00	9.50	0.00	17.25	1.17	1.00	50.96	3.06	0.10	30.58	16.50
									49.98			29.99	
3.20	15.00	2.00	25.00	9.50	0.00	17.25	1.17	1.00	50.96	3.06	0.10	30.58	16.00
3.20	20.00	2.00	25.00	9.50	0.00	17.25	1.17	1.00	51.94	3.12	0.10	31.16	16.00
3.20	25.00	2.00	25.00	9.50	0.00	17.25	1.17	1.00	51.94	3.12	0.10	31.16	16.00
									51.61			30.97	
3.20	30.00	3.00	25.00	9.50	0.00	17.25	1.17	1.00	51.94	3.12	0.10	31.16	16.00
3.20	35.00	3.00	25.00	9.50	0.00	17.25	1.17	1.00	51.94	3.12	0.10	31.16	16.00
3.20	40.00	3.00	25.00	9.50	0.00	17.25	1.17	1.00	51.94	3.12	0.10	31.16	16.00
									51.94			31.16	
3.20	45.00	4.00	25.00	9.50	0.00	17.25	1.17	1.00	51.94	3.12	0.10	31.16	16.00
3.20	50.00	4.00	25.00	9.50	0.00	17.25	1.17	1.00	52.92	3.18	0.10	31.75	16.00
3.20	55.00	4.00	25.00	9.50	0.00	17.25	1.17	1.00	51.94	3.12	0.10	31.16	16.00
									52.27			31.36	
3.20	60.00	5.00	25.50	10.00	0.00	17.75	1.21	1.00	51.94	3.12	0.10	31.16	16.00
3.20	65.00	5.00	25.50	10.00	0.00	17.75	1.21	1.00	52.92	3.18	0.10	31.75	16.00
3.20	70.00	5.00	25.50	10.00	0.00	17.75	1.21	1.00	52.92	3.18	0.10	31.75	16.00
							1.18		52.59			31.56	16.07

SECOND CONCENTRATION

Hz	TIME	DF CYCLE	PRESSURE FEED (PSI)	PRESSURE RETENTATE (PSI)	PRESSURE PERMEATE (PSI)	TMP (PSI)	TMP (BAR)	TIME FUNCTION (MIN)	PERMEATE VOLUME (ML)	PERMEATE FLOW RATE (L/H)	MEMBRANE AREA (M2)	PERMEATE FLUX RATE (LMH)	TEMPERATURE
3.00	0.00		25.00	9.50	0.00	17.25	1.17	1.00	32.67	1.96	0.10	19.60	15.50

11 APPENDIX D

General statistical and empirical calculations

D.1. Transmembrane pressure (TMP)

$$TMP\ (Bar)\ =\ \left(\frac{P_i\ +\ P_o}{2}\right) -\ P_p$$

P_i – inlet pressure (Bar)

P_o – outlet pressure (Bar)

P_p – permeate pressure (Bar)

D.2. Cross Flow Velocity (CFV)

$$CFV\ (m/s)\ =\ \frac{1.273\ \times Q}{d^2}$$

Q – flow rate (m^3/s)

d – pipe diameter (m)

D.3. Permeate Flux (J)

$$J\ (LMH) =\ \frac{Q}{A}$$

Q – flow rate (L/h)

A – membrane area (m^2)

D.4. Nominal Water Permeability

$$NWP_{20^\circ C}(LMH) = J_W\ \times\ TCF_{20^\circ C}$$

Jw – Clean water (WFI) permeate flux (LMH)

$TCF_{20°C}$ – Temperature correction factor at 20°C

D.5. Membrane Flux Recovery

$$Membrane\ flux\ recovery\ (\%) = \frac{L_P}{L_{P,0}} \times 100\%$$

L_p – NWP after cleaning

$L_{p,\,0}$ – NWP before cleaning i.e. before start of experiment

D.6. Protein Concentration (A_{280nm})

$$Concentration\ (mg/mL)\ = \frac{A_{280nm}\ \times\ L}{\varepsilon_{280}}$$

A_{280nm} = measured UV absorbance at 280nm

L = length of the curvette (cm)

ε_{280} = sedimentation co-efficient of HSA (5.8g/L)

D.7. Protein Rejection

$$Rejection\ (\%) = 1\ -\ \frac{C_p}{C_f} \times 100\%$$

C_p = concentration in permeate (mg/mL)

C_f = concentration in feed (mg/mL)

D.8. Protein Concentration (Gel Polarization Model)

$$C_{OPT}\ = \frac{C_G}{2.718}$$

C_{opt} = optimum protein concentration at which to complete ultrafiltration

C_g = gel concentration

APPENDIX E

Fitted response surface diagrams

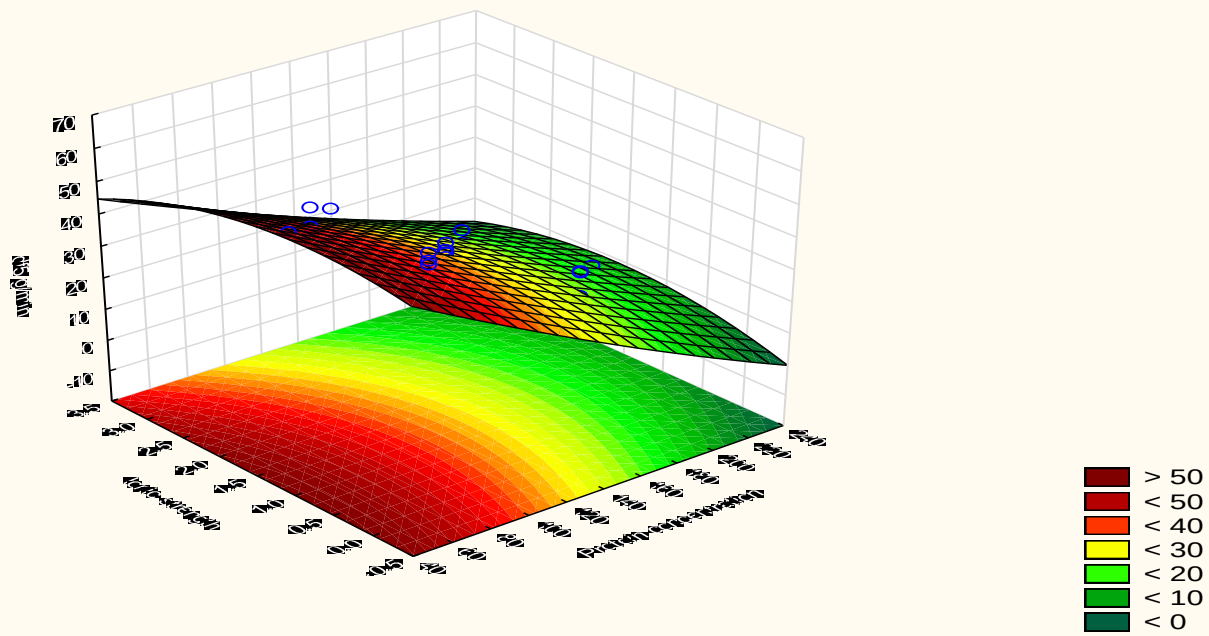


Figure E-1: Fitted surface response diagram for diafiltration step five showing protein concentration as a function of ionic strength and flow rate.

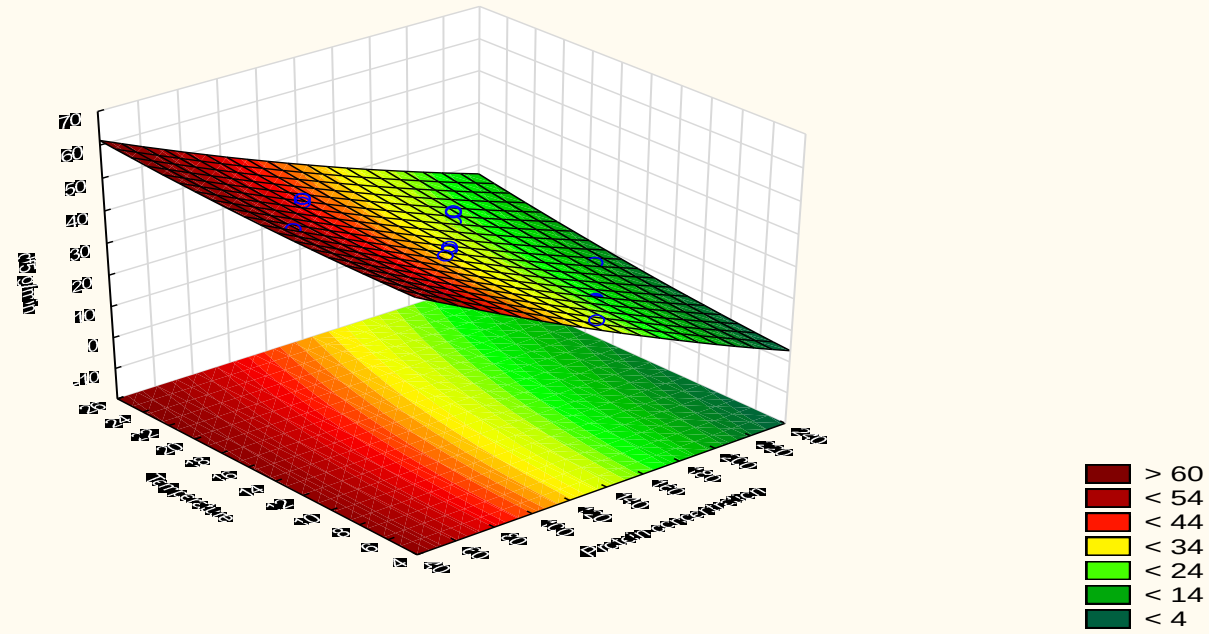


Figure E-2: Fitted surface response diagram for diafiltration step five showing protein concentration as a function of temperature and flow rate.

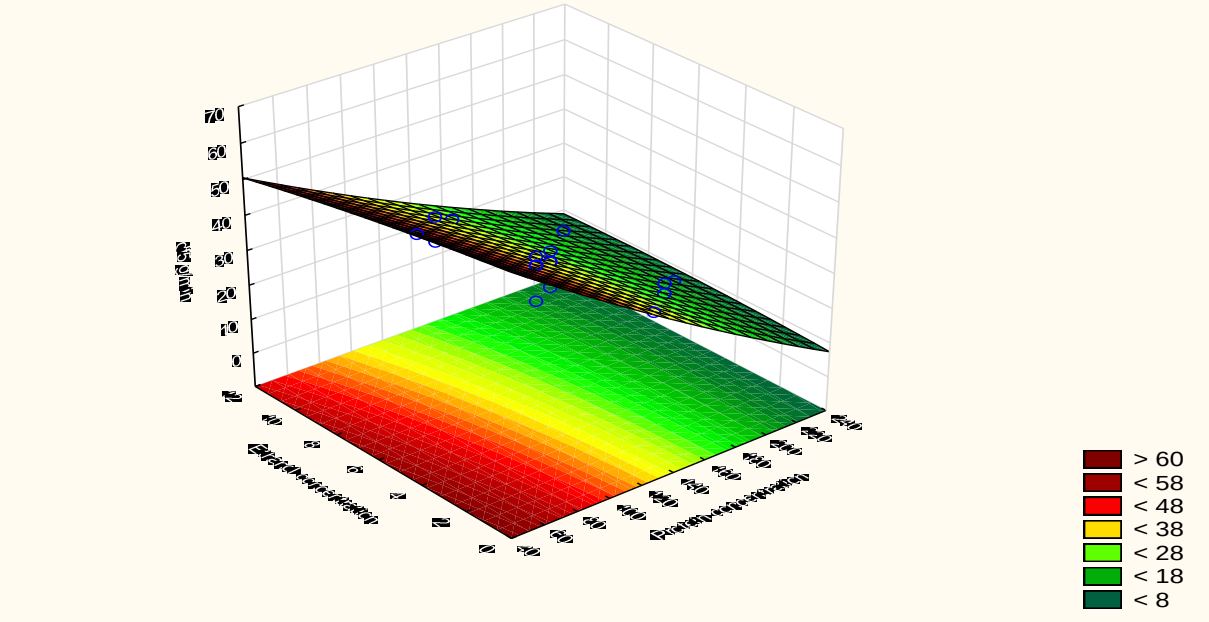


Figure E-3: Fitted surface response diagram for diafiltration step five showing protein concentration as a function of ethanol concentration and flow rate.

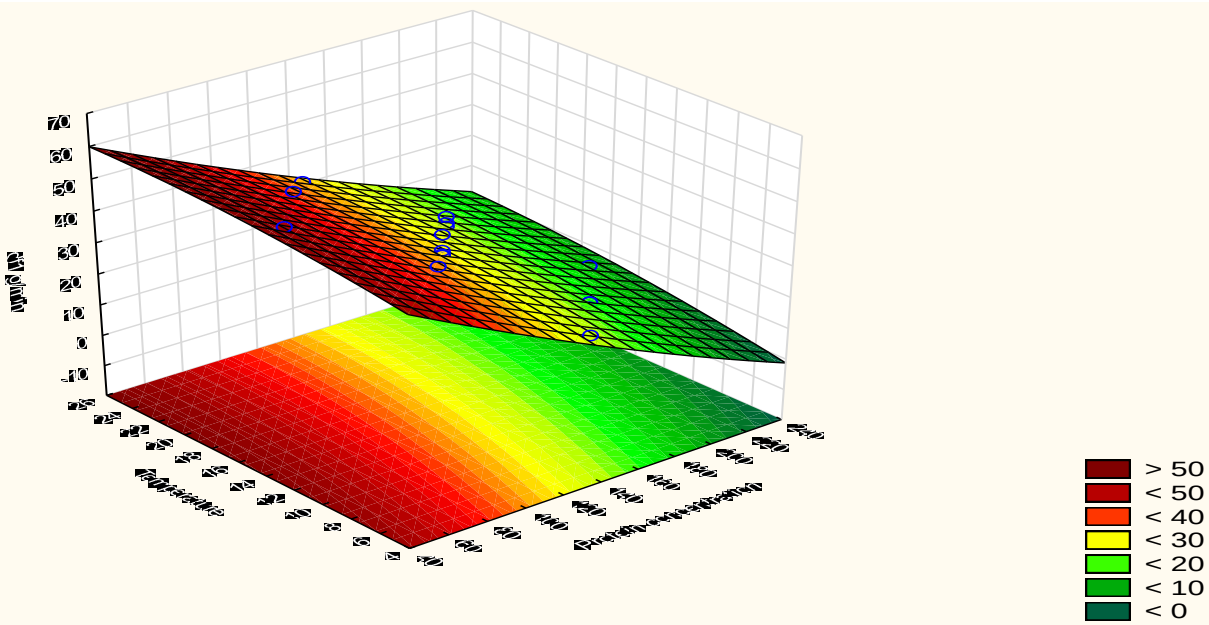


Figure E-4: Fitted surface response diagram for diafiltration step one showing protein concentration as a function of temperature and flow rate.

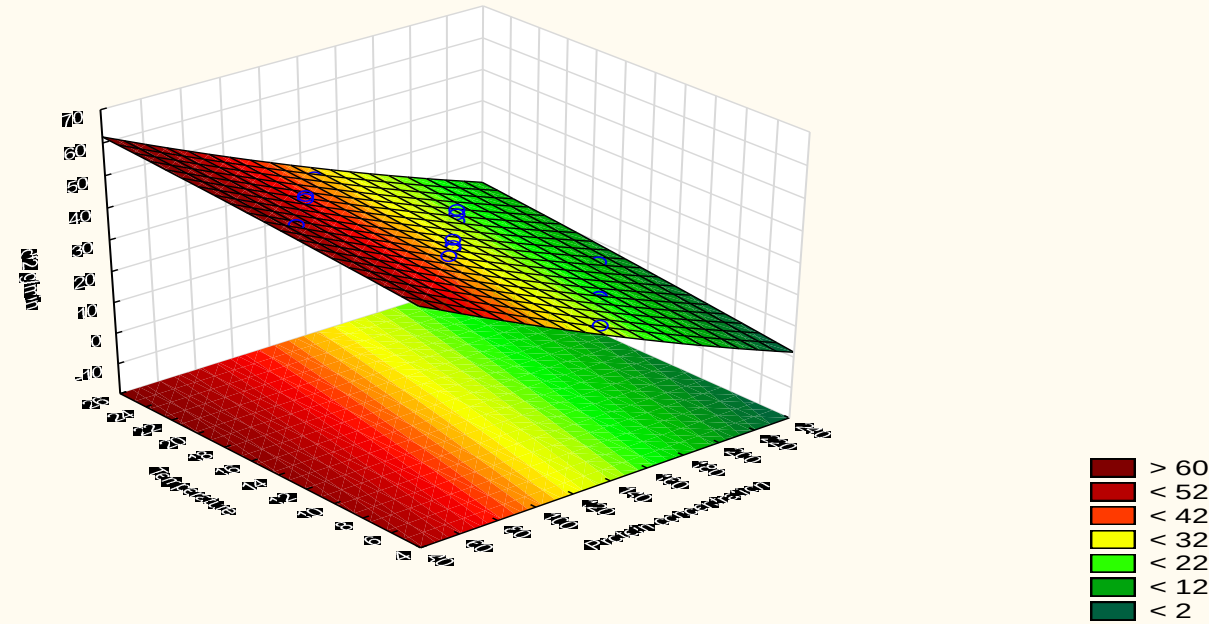


Figure E-5:: Fitted surface response diagram for diafiltration step 2 showing protein concentration as a function of temperature and flow rate.

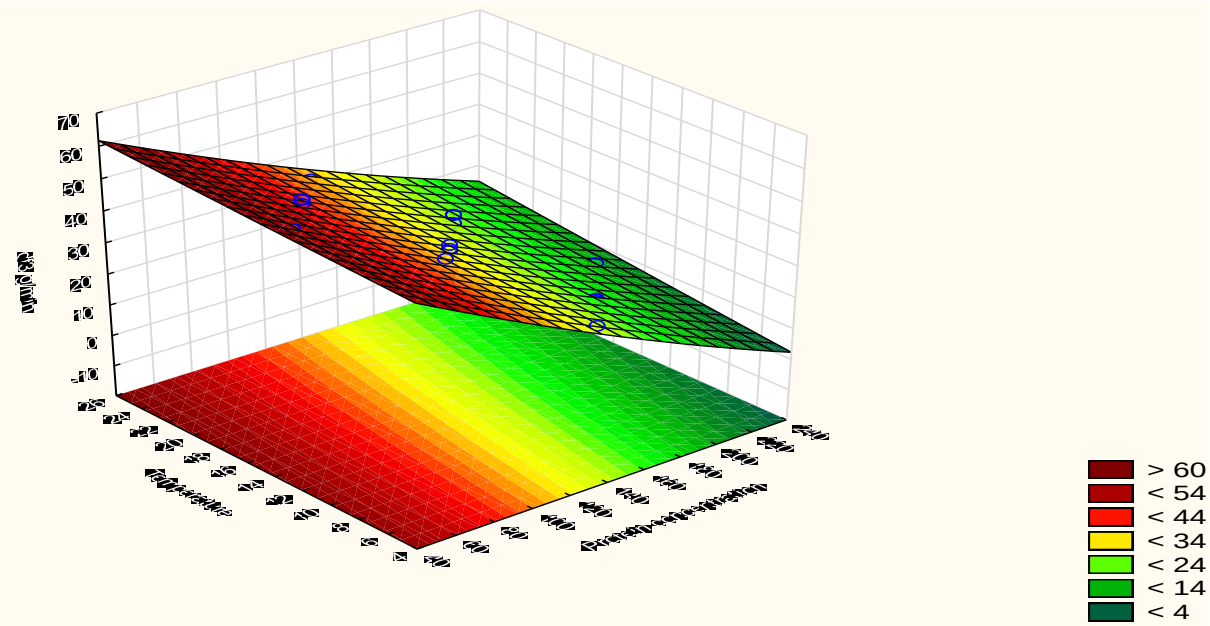


Figure E-6: Fitted surface response diagram for diafiltration step three showing protein concentration as a function of temperature and flow rate.

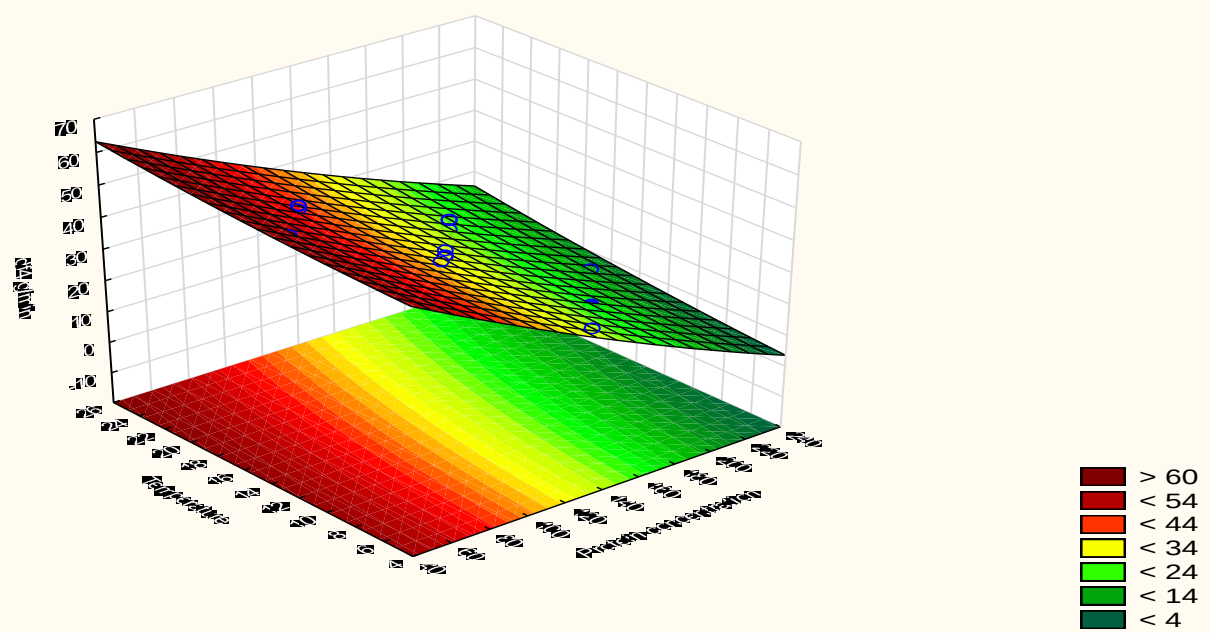


Figure E-7:: Fitted surface response diagram for diafiltration step four showing protein concentration as a function of temperature and flow rate.

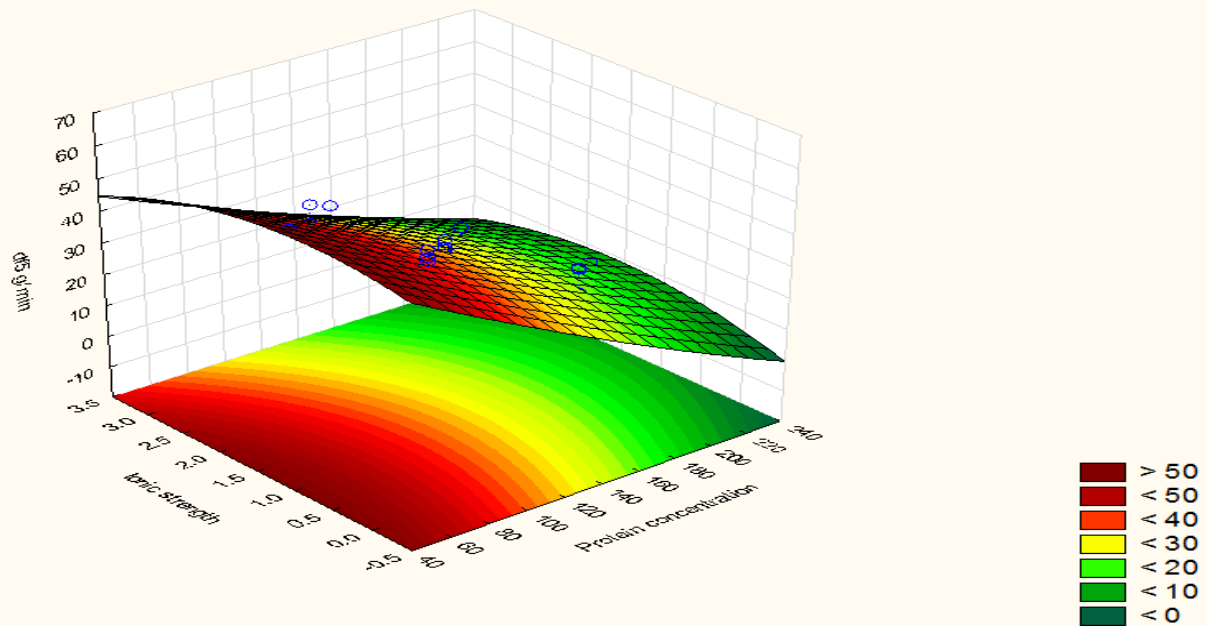


Figure E-8: Fitted surface response diagram for diafiltration step five showing protein concentration as a function of temperature and flow rate.

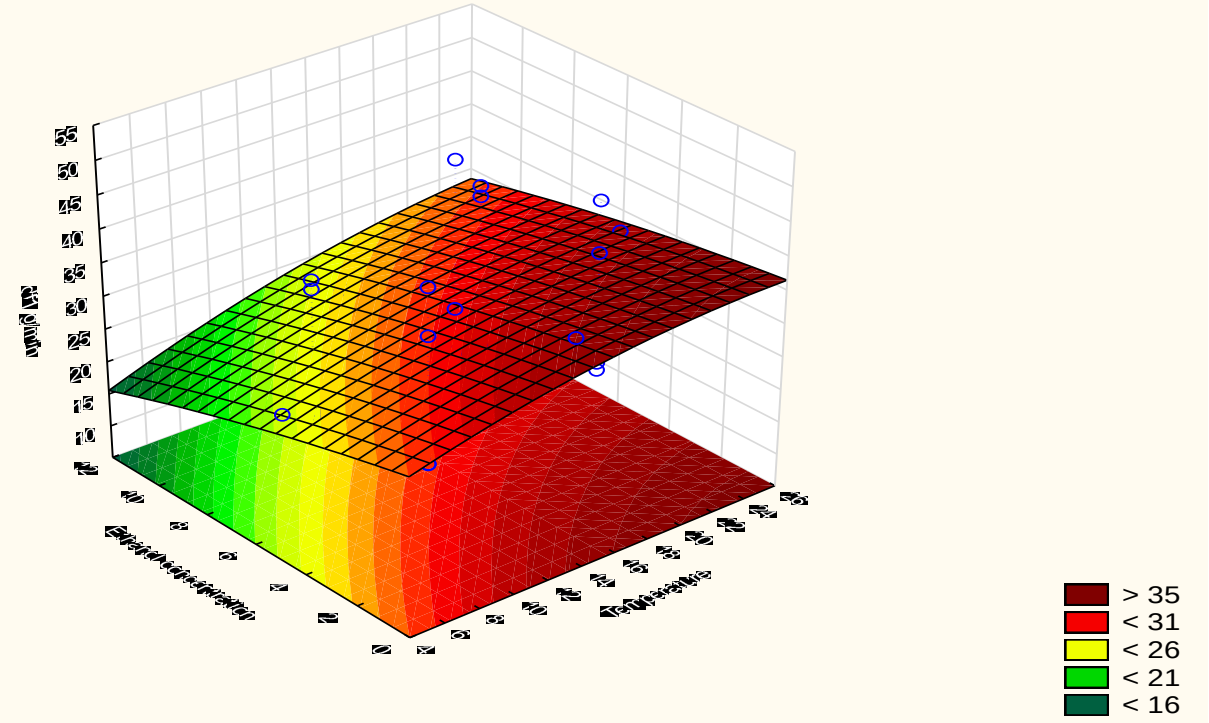


Figure E-9: Fitted surface response diagram for diafiltration step one showing temperature as a function of ethanol concentration and mass flow rate.

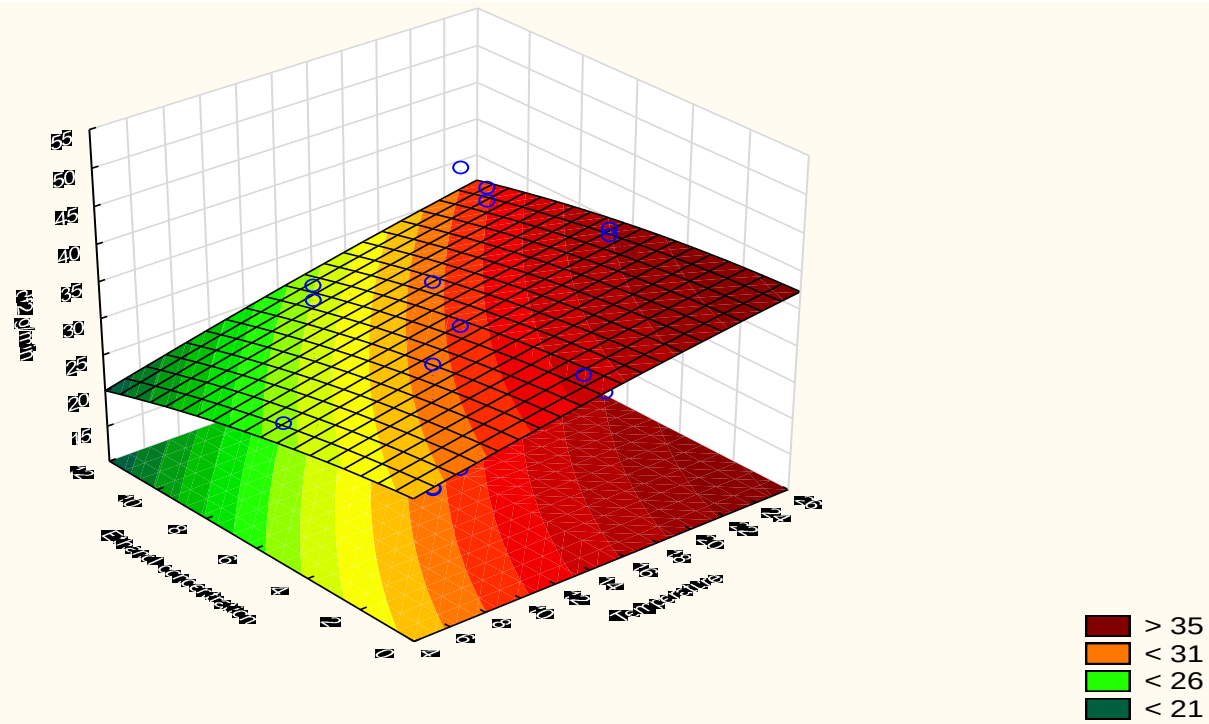


Figure E-10:: Fitted surface response diagram for diafiltration step two showing temperature as a function of ethanol concentration and mass flow rate.

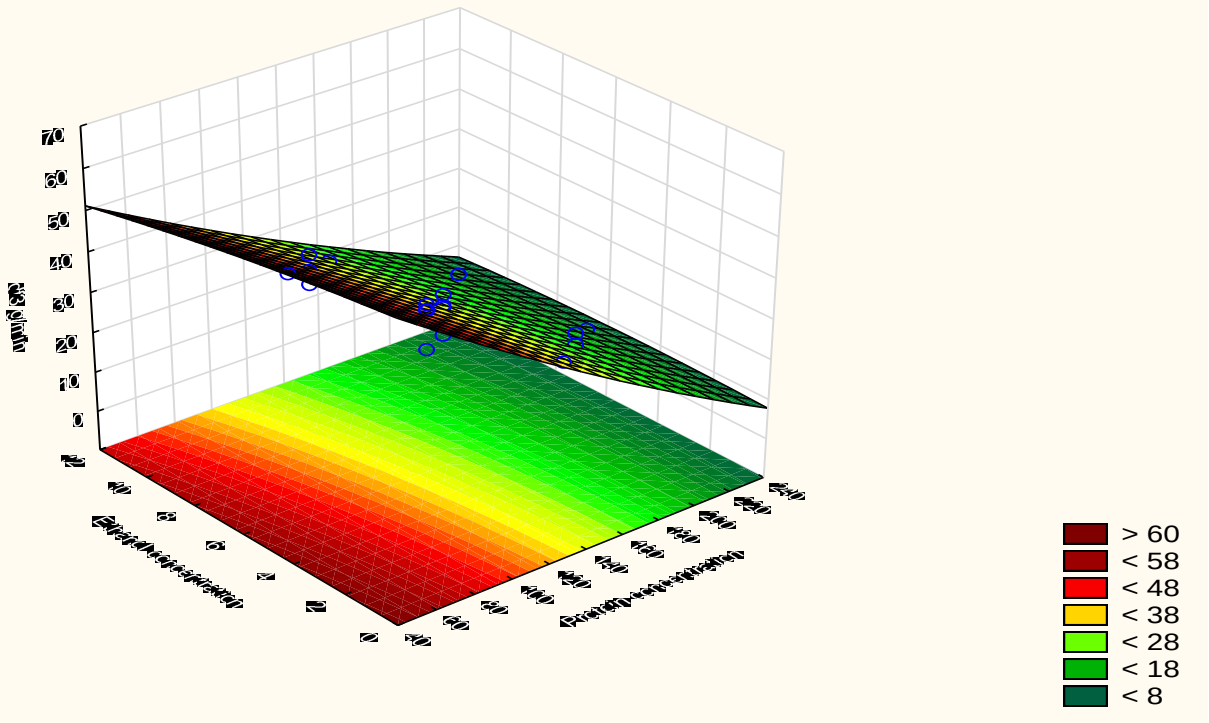


Figure E-11: Fitted surface response diagram for diafiltration step three showing temperature as a function of ethanol concentration and flow rate.

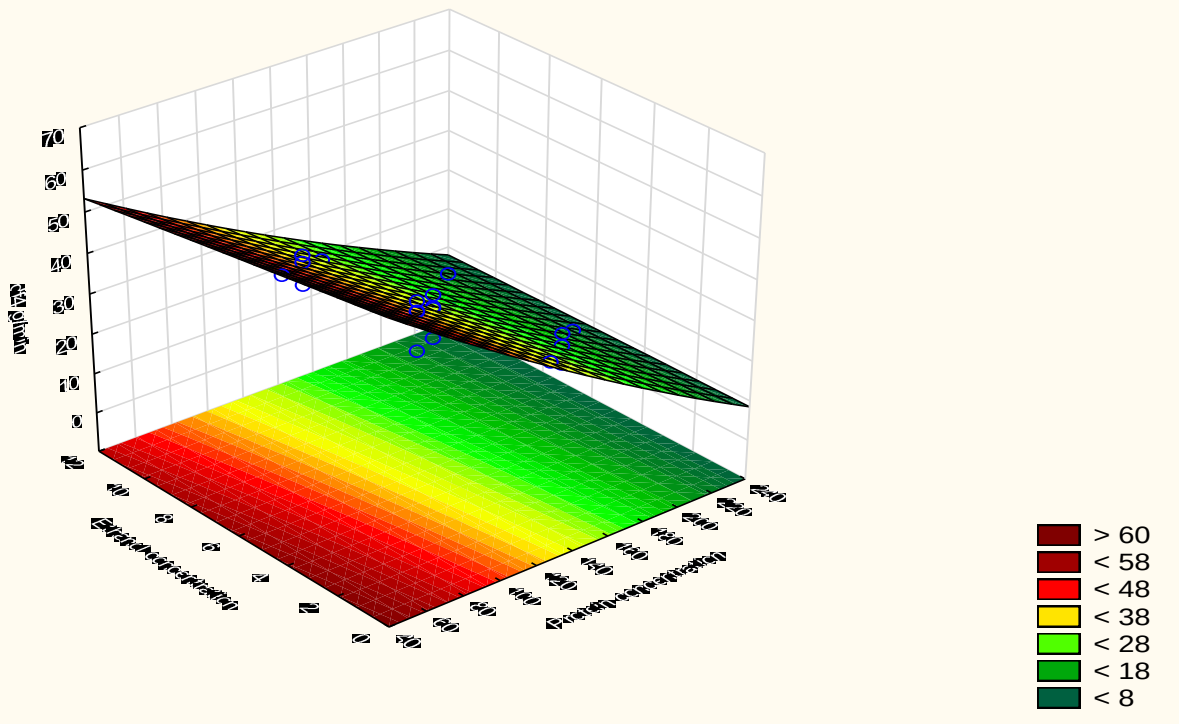
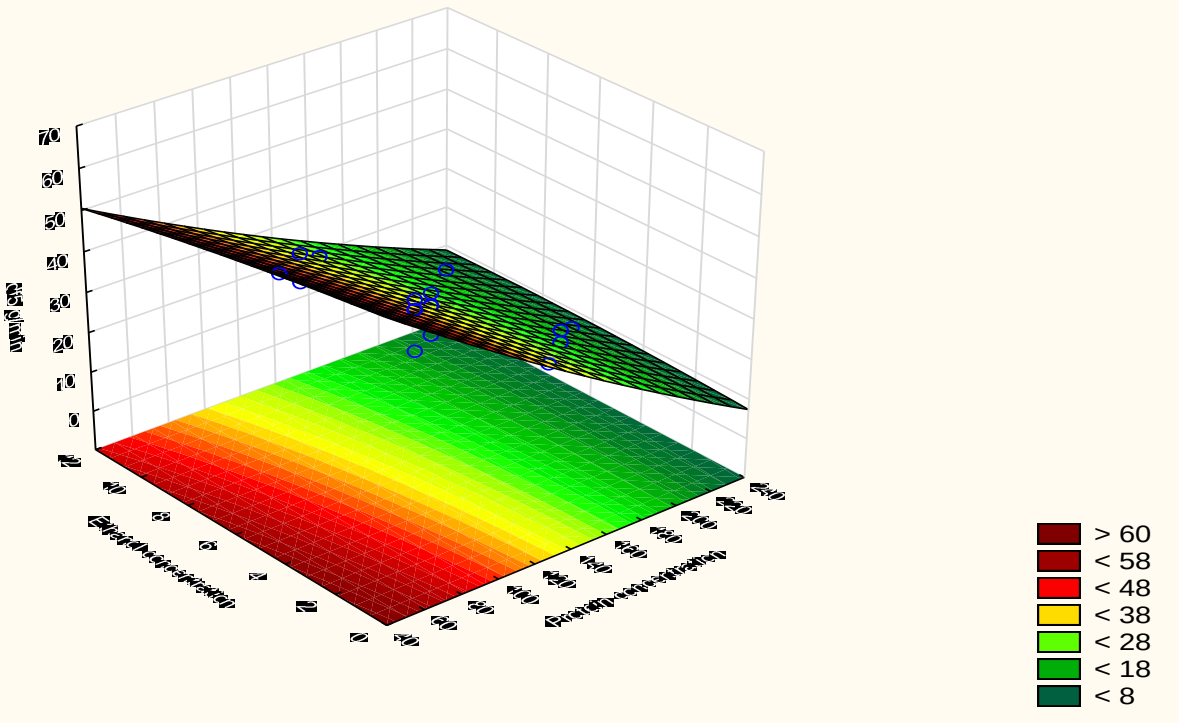


Figure E-12: Fitted surface response diagram for diafiltration step four showing temperature as a function of ethanol concentration and mass flow rate.



FigureE-13: Fitted surface response diagram for diafiltration step five showing temperature as a function of ethanol concentration and mass flow rate.